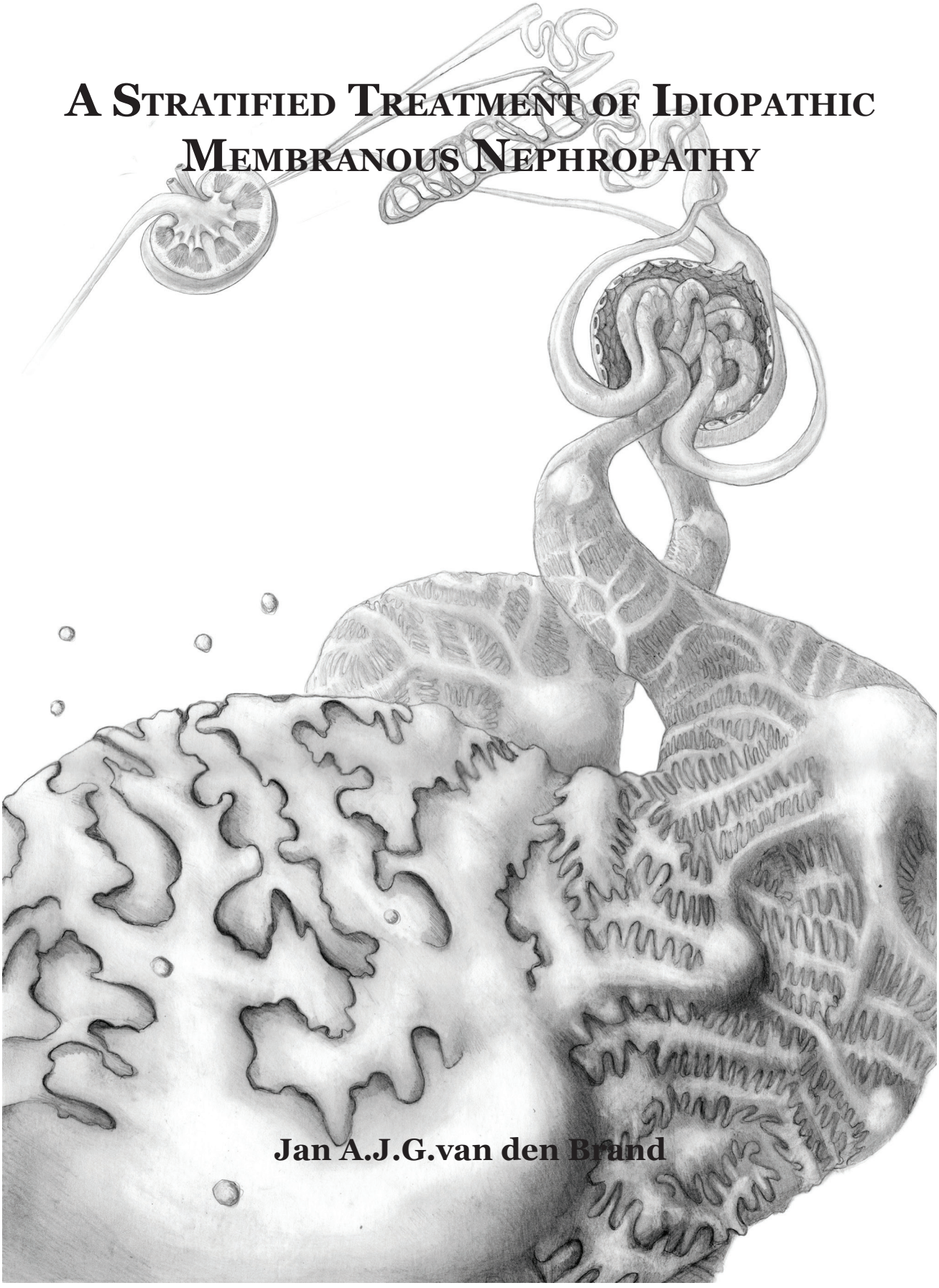
A Stratified Treatment of Idiopathic Membranous Nephropathy



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A STRATIFIED TREATMENT OF IDIOPATHIC MEMBRANOUS NEPHROPATHY

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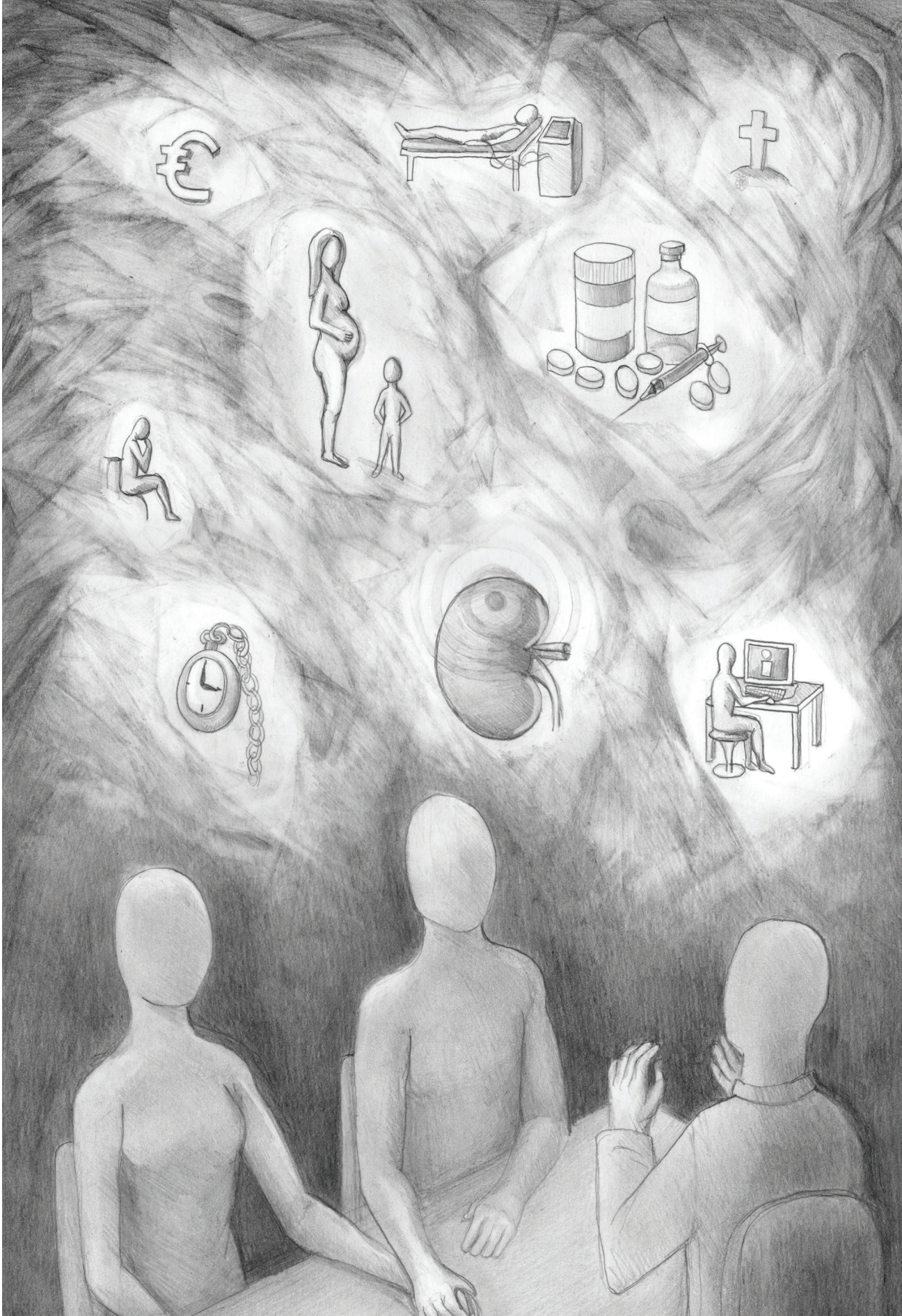

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Chapter 1: Introduction and Outline

Introduction

Nephrotic syndrome

A syndrome is a collection of symptoms which occur together and may point to a single underlying cause for these symptoms. In case of nephrotic syndrome the symptoms are massive protein loss via the urine (i.e. proteinuria of more than 3.5 grams per 24 hours), low serum protein level, in particular low serum albumin, and as a consequence severe edema and high serum lipid levels.[1] Due to loss of some serum proteins which have an anti-coagulatory function, the risk of thrombosis is increased in patients with nephrotic syndrome. Moreover, as antibodies are lost via urine, patients are at elevated risk of infections. Proteinuria, and thereby nephrotic syndrome is often a consequence of glomerular damage. When one of the components of the filtration barrier is damaged, its integrity is lost, and serum proteins pass into the pro-urine. In an attempt to salvage the proteins that are lost through the glomerulus, the tubular cells start reabsorbing more protein causing cell stress.[2] If the protein load is too high or goes on for too long, the tubular cells may die, causing more damage to the kidney. Ultimately, persisting nephrotic syndrome can result in end stage kidney failure. A patient then needs renal replacement therapy, such as dialysis or a kidney transplant.

Membranous nephropathy

Membranous Nephropathy is one of the most common causes of nephrotic syndrome in adults. Its incidence is estimated to be a little over one case per 100,000 persons per year in Caucasians.[3] Membranous nephropathy itself was introduced as the description of a histological pattern, characterized by thickening of the glomerular basement membrane found in the microscopic examination of a kidney biopsy. The thickened glomerular basement membrane is composed of IgG containing immune complexes that are deposited in the subepithelial space. Protrusions, or spikes, of the glomerular basement membrane that surround the deposits are often visible as characteristic spikes in silver stained biopsy slides as well. The IgG deposits active complement and cause podocyte injury with subsequent effacement of the foot processes. In approximately one third of the patients a clear underlying cause for the membranous nephropathy can be identified. This may be an infection, drug use, cancer or another underlying systemic disease.[4] In the remaining patients no underlying cause is found, and thus the membranous nephropathy is considered idiopathic –of unknown origin. In a rat model, membranous features were recreated.[5] This so-called Heymann nephritis was produced by immunizing rats with an extract from proximal tubules. Their immune systems subsequently produced antibodies against their own megalin, a transporter protein found in the cell membrane of the rat podocyte and proximal tubular cell. This led researchers to believe that idiopathic membranous nephropathy was an auto-immune disease. However, human podocytes do not express megalin. Consequently, the causative antigen in human idiopathic membranous nephropathy remained unknown for decades. Recently, Beck and colleagues showed that 70% of idiopathic membranous nephropathy

patients have circulating auto-antibodies against the M-type phospholipase A2 receptor (PLA2R).[6] This receptor is present on the human podocyte. Furthermore, a genome-wide association study found a link between a single nucleotide polymorphism associated with the PLA2R1 gene and the occurrence of idiopathic membranous nephropathy.[7] Together these findings suggest that anti-PLA2R antibodies are causative for idiopathic membranous nephropathy in a large proportion of patients, and that it is indeed an auto-immune disease. Nevertheless, as the disease is historically known as idiopathic membranous nephropathy and no antigen has been identified in approximately 30% of all patients, it will be referred to as such throughout this thesis, even though it may no longer be of unknown origin for many patients.

Personalized treatment

The natural course of idiopathic membranous nephropathy is quite variable. Patients who never develop nephrotic syndrome, i.e. their urine protein levels remain less than 3.5 g/day, almost always have a stable kidney function. Approximately 50% of the patients who do present with nephrotic syndrome will show a spontaneous remission of proteinuria and stabilization of kidney function.[8] The remaining patients will show progressive kidney failure and may ultimately require renal replacement therapy.[9] At present, renal replacement therapy, by dialysis or a kidney transplantation, is one of the most expensive treatments in healthcare. Therefore, membranous nephropathy may weigh heavily on the healthcare budget, despite its relative rarity.

As the presence of nephrotic syndrome and proteinuria are strongly linked to kidney failure, the initial treatment of idiopathic membranous nephropathy is aimed at reducing proteinuria. Since the 1990s angiotensin converting enzyme (ACE) inhibitors and angiotensin two receptor blockers (ARB) are being used as supportive care, as these drugs reduce proteinuria as well as blood pressure. However, historically immunosuppressive treatment has been used, as membranous nephropathy was assumed to be an auto-immune disease. For example, in the late 1970s patients were treated with steroids. Although a first trial with steroids appeared successful,[10] other studies failed to show a similar effect.[11,12] Later, a seminal trial performed by Ponticelli and colleagues showed that suppressing patients' immune system with chlorambucil, an alkylating agent used in cancer therapy, resulted in remission of proteinuria. Moreover, it reduced the risk of needing renal replacement therapy and overall mortality in treated patients compared to patients who received placebo.9 Later the same research group published a paper in which they showed that treatment with cyclophosphamide, another alkylating agent, gave similar results, but less severe side effects.[13] Nevertheless, cyclophosphamide is toxic. In particular, alkylating agents are associated with increased risk of infertility and cancer, and therefore their use remains debated to this day.

The trials underpinning the evidence for efficacy of these agents assume that all patients would be treated. However, given the variable disease course, up to half of all patients could thus be unnecessarily exposed to these toxic drugs. Therefore, restrictive use of immunosuppression has been advocated.[14] Indeed, the recently published Kidney Disease: Improving Global Outcome (KDIGO)

guidelines recommend that all patients be treated with supportive care, and they only recommend immunosuppressive therapy in patients suffering nephrotic syndrome for at least six months, when kidney function deteriorates, or when severe symptoms of the nephrotic syndrome are present.[1] However, there is very little direct evidence to support the recommendation for restrictive treatment. Moreover, long term consequences are largely unknown. Furthermore, restrictive, personalized therapy requires accurate prediction of prognosis, so that only patients who really need immunosuppressive therapy receive it, and patients who are likely to show a sponteneous resmission are spared from side effects.

Predicting prognosis

The past decades of research in idiopathic membranous nephropathy have been marked by the search for prognostic markers to identify high risk patients.[15-18] To this day, a sustained decrease in glomerular filtration rate has proven to be the most powerful marker of a poor prognosis.[19] Conversely, remission of proteinuria, either spontaneous or after treatment, is associated with favorable outcome.[20] Unfortunately, a decrease in glomerular filtration rate or remission of proteinuria requires a long follow-up period, leaving the patient and physician in uncertainty. Furthermore, a decrease in glomerular filtration rate may be insidious. Delayed start of immunosuppression has been associated with higher complication rates and reduced efficacy, as irreversible damage may have already occurred.[21,22] Other validated prognostic markers are used as well. However, these need reassessment as the clinical course of the idiopathic membranous nephropathy has changed since their introduction, mostly due to the now universal use of ACEi/ARBs.[23,24]

The balancing act

In summary, cyclophosphamide therapy is effective in idiopathic membranous nephropathy. However, it can give severe side effects, and therefore restrictive treatment is recommended. Unfortunately, evidence supporting restrictive regimens is lacking. Moreover, the magnitude of risks associated with cyclophosphamide therapy in patients suffering from idiopathic membranous nephropathy is not well known. As a consequence, balancing the benefits and risk of therapy is difficult, both at the individual patient level and at the societal level.

Thesis outline

Given the background of idiopathic membranous nephropathy and its treatment, the following questions arise:

1. How well can we predict prognosis and identify at high risk of progression in idiopathic membranous nephropathy?
2. Can we improve prediction of prognosis?
3. Is restrictive therapy with cyclophosphamide effective?
4. What in the malignancy risk of cyclophosphamide therapy?
5. Do the benefits of restrictive cyclophosphamide therapy outweigh the risks and costs?

In order to answer these questions, epidemiological studies and a decision analysis have been performed. The studies in this thesis build on previous work.

Together with allied centers from the South and East of the Netherlands, the Radboud university medical centre has established a registry and biobank of patients with glomerular diseases. Urine and blood samples of these patients were obtained and stored in a standardized fashion. In Chapter 2 we give a brief overview of the methodology used in this thesis and describe the Glomerular Disease Registry and the standardized protocol according to which blood and urine samples have been obtained.

Branten et al. described how urinary β_2-microglobulin excretion can be used to identify patients with idiopathic membranous nephropathy at the highest risk of progression.[24] However, as the procedures to measure urinary β_2-microglobulin are cumbersome, the search for other markers continues. Alpha-1-microglobulin has been proposed as such a marker. Furthermore, despite validation, prognostic markers often do not live up to their expectations when introduced into clinical practice. For example, since the introduction of urinary β_2-microglobulin into clinical practice, therapeutic blood pressure targets have been lowered, and ACEi and ARBs have become standard care. This may have altered the disease course of patients who have not been treated with immunosuppressive drugs. The predictive performance of urinary β_2-microglobulin may have changed as a consequence. Therefore, in chapter 3.1, we evaluated the prognostic value of urinary β_2-microglobulin as well as α_1-microglobulin in current clinical practice.

International guidelines for the diagnosis and treatment of idiopathic membranous nephropathy recommend a prognostic algorithm as an alternative to urinary β_2-microglobulin.[1] This algorithm, developed and validated by Cattran and colleagues,[18,23] uses the minimal total urinary protein excretion over a six month period of maximal proteinuria in combination with the initial creatinine clearance and the change in creatinine clearance during that six month time frame. Compared to measuring β_2-mciroglobulin, it requires a more prolonged follow-up. Which of the two prognostic strategies is most accurate has not been evaluated. We performed a head-to-head comparison of the urinary low molecular weight proteins α_1- and β_2-microglobulin and the prognostic algorithm by Cattran and colleagues, which we named the Toronto Risk Score. This study is described in chapter 3.2.

Once prognosis has been predicted, patients are monitored closely. Should complications of the nephrotic syndrome arise or if their kidney function shows signs of deteriorating, immunosuppressive therapy with cyclophosphamide is advised.[1] Otherwise, patients are treated with ACEi and/or ARBs to control blood pressure and reduce proteinuria. This treatment strategy, recommended in the KDIGO guidelines, has already been used at the Radboud university medical centre and allied centers from 1995 onward. Now, over fifteen years later, we are in a position to evaluate the long term outcomes of this treatment strategy. Chapter 4.1 describes rates of mortality and renal replacement therapy, being dialysis or kidney transplantation, as well as the occurrence of adverse events.

Even though alkylating agents, and especially cyclophosphamide, have been shown to be effective in the treatment of idiopathic membranous nephropathy,[9,13,22,25] their use is not universal nor without criticism. Cyclophosphamide has long been used as chemotherapy in non-Hodgkin's lymphoma, rheumatoid arthritis and anti-neutrophil cytoplasmic antibody (ANCA) associated vasculitis. It has been reported to increase the long term

risk of malignancy in exposed patients.[26-28] However, data from patients with other diseases cannot necessarily be extrapolated to patients with idiopathic membranous nephropathy. Furthermore, to balance the risk and benefits of cyclophosphamide therapy, an estimate of the magnitude of malignancy risk in treated idiopathic membranous nephropathy patients is required. Therefore, in chapter 4.2, we evaluated the incidence of malignancies in patients with idiopathic membranous nephropathy who were treated with cyclophosphamide compared to the incidence in untreated patients.

Finally, in Chapter 5, the risks of progressive kidney failure, renal replacement therapy, death, malignancy and other complications and the benefits of universal or restrictive cyclophosphamide therapy are brought together in a Markov model. Additionally, we estimated healthcare related costs associated with possible treatments and outcomes. The model is to aid policy makers as well as individual physicians and patients in making a personalized and well informed decision about an optimal treatment strategy.

We provide a summary of the findings in this thesis in chapter 6. In addition, we put our findings into perspective with recent advances in the field of idiopathic membranous nephropathy. Chapter 7 provides a Dutch language summary of the main findings presented in this thesis.

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Chapter 2: Population and Methods

Abstract

This chapter describes study methods commonly used throughout this thesis. First an overview of the standardized and timed urine analysis and laboratory procedures is given on which the first two studies in the thesis are based. Second, the data collection for the biobank and patient registry created at the Radboud university medical centre is described. All studies presented in this thesis are based on data from this registry. The final section provides an overview of the epidemiologic and decision analytic methods used in the following chapters. This last section is intended as a basic introduction for the reader who is unfamiliar with the conduct and/or analysis of epidemiologic studies or decision analysis, respectively.

Standardized timed urine analysis

Since 1995 patients suffering from the nephrotic syndrome are referred to our clinic, either by family doctors in our catchment area or by allied internal medicine and nephrology clinics. These patients undergo a timed urine analysis. To date, over 1000 patients suffering from glomerulonephritis have attended. The goals of the timed urine analysis are twofold, on the one hand results are used to predict prognosis, and support clinical decision making. On the other, samples and data collected are used for scientific studies to improve the treatment of various forms of glomerulonephritis.

The standardized timed urine analysis has been described previously.[1,2] In summary: prior to undergoing the urine analysis, patients are asked to fast overnight. In addition, they are instructed to collect two 24 hour urine samples in the days before the visit. Furthermore, patients take 4000 milligrams of sodium bicarbonate on the evening before attending our clinic to ensure that urinary pH exceeds 6.0, as β_2-microglobulin degrades in acidic urine. Patients are not allowed to take any diuretics on the morning of the measurement. Other medications are taken as usual. Upon arrival to the clinic, patients are given 375 to 500 milliliter of tap water to enforce diuresis. Patients remain supine for one hour except for voiding. Blood pressure is measured ten times with an interval of 5 minutes using an automated device (Dinamap, Critikon, Tampa FL, USA). In the middle of the urine collection period, a blood sample is drawn.

In the blood samples, sodium, potassium, urea, creatinine, calcium, phosphate, hemoglobin, hematocrite, cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol, albumin, IgG, transferrin and β_2-microglobulin levels are determined. Likewise, sodium, potassium, creatinine, total protein, albumin, IgG, transferrin, α_1- and β_2-microglobulin concentration are measured in the timed urine samples. Sodium, urea, total protein and creatinine levels are determined in the 24 hour urine samples. Most concentrations are measured using standard automated techniques. Concentrations of α_1-microglobulin, albumin, transferrin, IgG are measured by immunonephelometry using on a BNII nephelometer (Behring, Marburg, Germany). Beta-2-microglobulin concentrations were measured using an ELISA.[2] In addition, blood and urine samples for every patient are taken and stored at -80 °C.

The glomerular disease registry

Parallel to the standardized urine analysis a prospective registry of referred patients with glomerulonephritis is kept. At the visit for standardized urine analysis, demographic parameters such as date of birth, ethnicity and gender are registered. Additionally, the date of biopsy, biopsy identification number for the PALGA registration and the names of the referring centre and physician are recorded.

Follow-up data are collected periodically from medical records at the referring centers. Blood pressure and weight are recorded from out-patient records. In addition, laboratory data including serum creatinine, albumin, urea and cholesterol

as well as urinary protein and creatinine concentrations are obtained. Prescribed ACE inhibitors, ARBs, diuretics and other antihypertensive medication, lipid lowering medication, anti-coagulation and non-steroidal anti-inflammatory drugs are recorded as well. Finally, immunosuppressive treatment regimens are registered. For cyclophosphamide the total cumulative dose per treatment episode is included as well. Recorded complications during follow-up include infections, fever, liver function abnormalities, malignancies, incident cardiovascular disease, cerebrovascular accidents, embolism, leucopenia and thrombopenia. If a patient develops end stage kidney disease during follow-up, this is recorded as well. Finally, upon death of a patient, the date and cause of death as reported by the treating physician are registered.

Study designs

The following section provides a brief introduction on the research designs and analyses presented in this thesis. This section is aimed at readers who are unfamiliar with epidemiologic research and/or economic evaluations in healthcare.

Predictive studies

The ultimate goal of predictive studies, either diagnostic or prognostic, is to accurately predict the occurrence of an event.[3] Events may be the presence or onset of a disease or the occurrence of a long term outcome, such asrenal replacement therapy. The gold standard design for predictive studies is a cohort. Patients are included at similar time in the course of their disease. Subsequently a test is performed to predict either diagnosis (having a disease right now) or prognosis (having a disease or other event sometime in the future). The example in table 1 shows such a test. In this example body temperature is measured to establish the presence of an infection. At each 0.5 °C increase of body temperature a threshold for test positivity can be placed, six in total. Using the resulting test characteristics, a receiver-operating characteristics (ROC) curve can be plotted.[4] For each threshold, the ROC curve plots the true positive rate (sensitivity) against the false positive rate (1 – specificity) at each threshold. Subsequently, an area under the ROC curve can be calculated. An area of 1 signifies perfect discrimination, and thus

Table 1. Body temperature in persons with and without an infection.

Temperature	Number of diseased persons	Number of undiseased persons	Risk of infection
≥38.0	13	1	93%
37.5-37.9	8	5	62%
37.0-37.4	4	19	17%
36.5 -36.9	2	25	7%
<36.5	1	12	8%
Total	28	72	

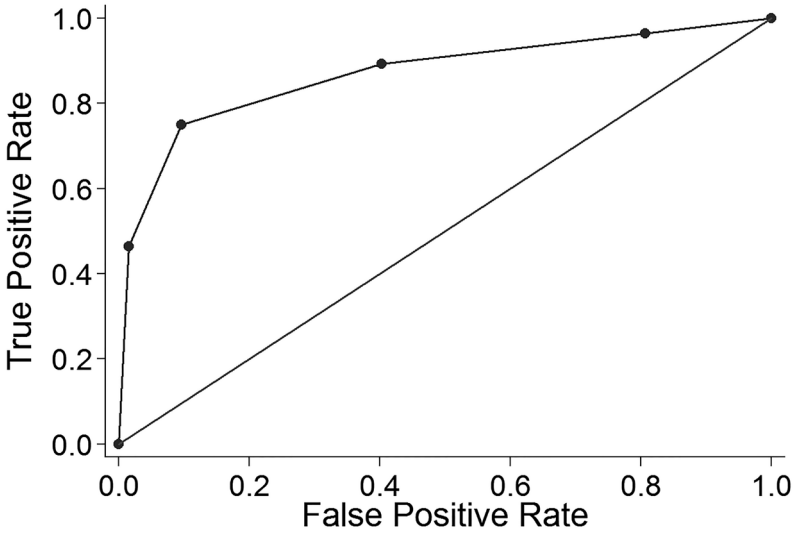

Figure 1. Receiver-operating characteristics curve for body temperature as a test for infection. The diagonal reference line signifies an uninformative test, such as tossing a coin. See table 1 for the data on which the curve was based. The point estimates from left to right mark the following thresholds: classifying none as diseased, ≥38.0, ≥37.5, ≥37.0, ≥36.5 and classifying all patients as diseased.

a prefect test. An area of 0.5 is similar to tossing a coin, completely random, and thus a useless test. Figure 2.1 shows the ROC curve for body temperature as a test for infection, based on the data in table 1. The area under the curve of 0.87 means that if we are faced with two patients and measure body temperature, we will correctly point out the person who is at highest risk of having an infection 87% of the time. Note that ROC analysis only gives ranks and does not provide information on the magnitude of difference in risk. Furthermore, whether an area under the ROC curve of 87% provides sufficient discrimination depends on the consequences of the decision. For example, in idiopathic membranous nephropathy, patients may be treated with toxic drugs. In this case we would like to minimize the number of patients with a false positive test result. However, we do not want to wait with treatment until irreversible damage has occurred. There is very little leeway in this decision, and thus a very high discriminatory power is required.

Rather than a just a single test variable, numerous possibly predictive variables can be related to an event of interest using a regression model, such as logistic or Cox regression.[4] Conceptually, a regression model is the lump sum of many simultaneous tests and can be viewed as a test itself. It yields a dimensionless score which can be recalculated into the predicted probability of the event occurring.

Subsequently, the observed probability of the event can be plotted against its predicted probability, which is based on the test, for several groups of patients divided by their predicted probability of the event.[4] In the example patients have been categorized in five groups with increasing predicted risk of an event. Their actual risk is show in table 1. In the case of optimal calibration, the observed and predicted probabilities closely match. It means that persons with a predicted high

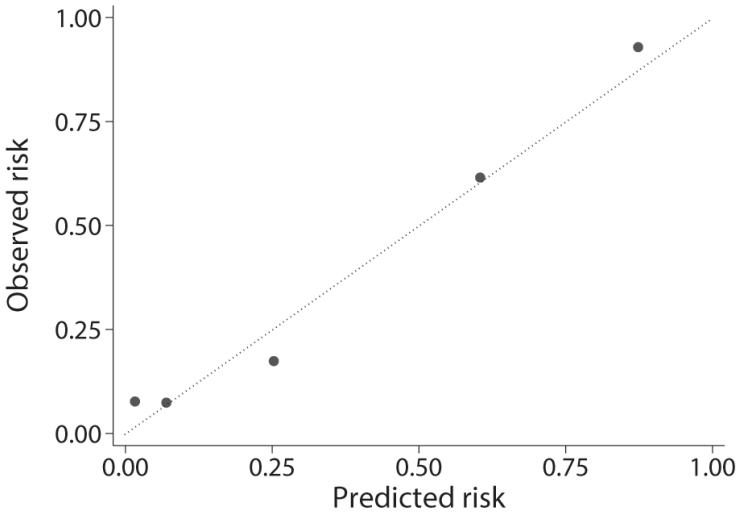

Figure 2. Calibration plot for body temperature as a test for infection. An ideal test closely fits the diagonal line.

risk are at a substantially higher risk of the event compared to low risk patients. However, if the observed (i.e. actual) probability of the event increases little with a marked increase in the predicted chance of an event, the test may not be very helpful –even if discrimination is good. In that case an increase in test result does not result in any clinically relevant difference in risk. Figure 2.2 shows the calibration plot for our body temperature example. Here we can see that the actual risk of infection increases with predicted risk of infection based on body temperature. However, in the lower ranges of predicted risk, calibration is rather poor. At levels lower than 37.5 °C, the observed risk of an infection increases very little despite increasing body temperature. Therefore, we can conclude that our test is only clinically useful at higher body temperatures.

Following the creation of a test or a predictive model, it needs to be validated.[5] This is done using the same design and similar analysis methods, but in different patients. The most accepted and valid method is using a different cohort of contemporary patients. Another option is temporal validation, in which a new cohort is created after the test has been introduced. Other options such as internal validation may be useful, but are considered inferior to external validation. [5]

For further reading on the subject, consider the excellent treatises by Altman, Royston, Moons and Vergouwe.[3-6]

Etiologic studies

In etiologic studies the goal is to provide an accurate, unbiased description of the association between an exposure and subsequent disease or outcome. Basically, a population is followed for a period of time and during this time events occur. Subsequently, if we have a dichotomous (yes/no) exposure, like in chapter 6 where patients were either treated with cyclophosphamide or not, the number of events can be divided by the total amount of follow-up time accrued by all patients in

either exposure group. This is called the incidence.[7] Then a ratio of incidences can be calculated to investigate the effect of a certain exposure on the outcome of interest. In a second paper VandenBroucke and Pearce give an insightful example, a slightly adapted version will be presented here.8

As a thought experiment we will consider the possible effect of oral contraception use on the occurrence of deep venous thrombosis in women of a reproductive age. Say we have a dynamic population of 1.2 million women of a reproductive age during the year 2012, one thirds of whom use oral contraceptives. Furthermore, we assume that the population is in a steady state. In other words, we assume that the number of women in either exposure groups stays virtually the same over the year. This is often a realistic assumption.[7] Subsequently, we observe 400 new cases of deep venous thrombosis within the oral contraceptive using women and 200 in the unexposed women during the year. The resulting incidence in the exposed women is 400 / 400,000 = 10 per 10,000 women years. Likewise, the incidence in the unexposed women in 200 / 800,000 = 2.5 per 10,000 women years. Consequently the incidence ratio equals 4.0, which means that women who use oral contraceptives are four times more likely to have deep venous thrombosis compared to unexposed women. The precision with which this incidence ratio is estimated can be expressed as a 95% confidence interval. Here the confidence interval ranges from 3.4 to 4.8, which means that we can say with 95% certainty that upon repeating the study in a similar population an incidence ratio between 3.4 and 4.8 will be found. Or, in simpler, broadly correct terms: the 'true' incidence ratio for deep venous thrombosis due to oral contraceptive use most likely lies between 3.4 and 4.8. We now know that if we were to remove oral contraceptive use, the incidence of deep venous thrombosis in our hypothetical population would decrease. Essentially, this is the goal of etiologic studies. Finding exposures that result in disease so that we may be able to prevent or treat the disease by intervening on that exposure.

If there are no other factors related to both the exposure and outcome, the calculations described above result in an unbiased effect estimate of the exposure on the outcome. This is the case when an investigator randomly assigns the exposure, as is done in clinical trials. However, in an observational setting, potential risk factors for disease often occur in clusters. This makes attributing differences in incidence to a single exposure murky business. In other words, the association between the exposure and outcome of interest may be confounded. One way to deal with such confounding is to perform stratified or standardized analyses. However, regression analysis, such as logistic or Cox regression, is more efficient when many confounders act simultaneously. Please note, that although regression analysis is used in predictive studies as well, the goals of an etiologic study are completely different. Rather than optimizing a prediction, the regression is used to remove confounding bias from the estimated incidence ratio for the exposure of interest on the outcome. Therefore, only an incidence ratio is reported, adjusted for the confounding effects of other risk factors if need be, and no mention is made of ROC curves or calibration plots.

Medical decision making and economic evaluation

The previously described research methods fall within the realm of epidemiology, whereas decision models are considered health economics and are a part of health technology assessment. As such, this study design is the odd one out within this thesis. Nevertheless, health technology assessment, like epidemiology, studies and aids medical decision making, albeit more directly. In chapter 5 a cost-effectiveness analysis has been performed. In essence, the goal of a cost-effectiveness analysis is to make explicit the possible benefits, costs, risks and uncertainties underlying a decision and weigh these in order to choose the optimal course of action from two or more alternatives. The perspective from which a decision is being made impacts the inputs for the cost-effectiveness analysis.[9] For instance, if a patient is making the decision, only costs for the patient him or herself are relevant. Likewise, the quality of life associated with possible outcomes should be rated by the patient. Conversely, when a healthcare perspective is chosen, total healthcare costs and not just costs made by the patient are relevant. Moreover, for an analysis from a societal perspective productivity losses and other indirect costs (and benefits) need to be estimated. Similarly, in both the healthcare and societal perspective the general population, instead of the patient, is asked to value health states, as society as a whole is paying for the consequences of a decision. Costs can be determined bottom up by obtaining and summing the costs of, for example, each drug, intervention, taxi ride and day of absenteeism for each patient. Conversely, a top down calculation can be performed using averages from healthcare budgets or reimbursement claims.

Possible health states which are the consequences of a decision can be described in terms of health utility. Utility is a measure for the preferences of health outcomes ranging from 0 (death) to 1 (perfect health). To compare relative utility of different outcomes, quality adjusted life years (QALYs) can be calculated. A single QALY is the equivalent of a life year in perfect health. Utility scores for health states can be obtained by asking subject to rate a health state on a visual analog scale, with a standard gamble or through a time trade off. Each has its pros and cons, as discussed by Hunink and Glasziou.[9]

Both dimensions of cost-effectiveness can be brought together as the ratio costs per QALY gained. Subsequently, various alternatives can be compared by means of an incremental cost-effectiveness ratio. This is calculated by dividing the difference in costs by the difference in effectiveness for two alternatives. Unfortunately, both costs and effectiveness of the alternatives are often estimated with an amount of uncertainty. This uncertainty can be expressed in a cost effectiveness plane, as shown in figure 2.3.[9] Despite a greater effectiveness, a treatment may not be acceptable if it is far too costly. The constraints of healthcare budgets can be formulated as a willingness-to-pay threshold. At present, we as a society are willing to pay €80,000 for a QALY gained.10 Anything less efficient is generally not accepted. One can plot the probability that a certain alternative would be cost-effective by varying thresholds for the willingness-to-pay, giving a cost effectiveness acceptability curve.[11] Such a plot is useful when comparing cost-effectiveness across health systems.

Average outcome and costs for alternative strategies are obtained from decision

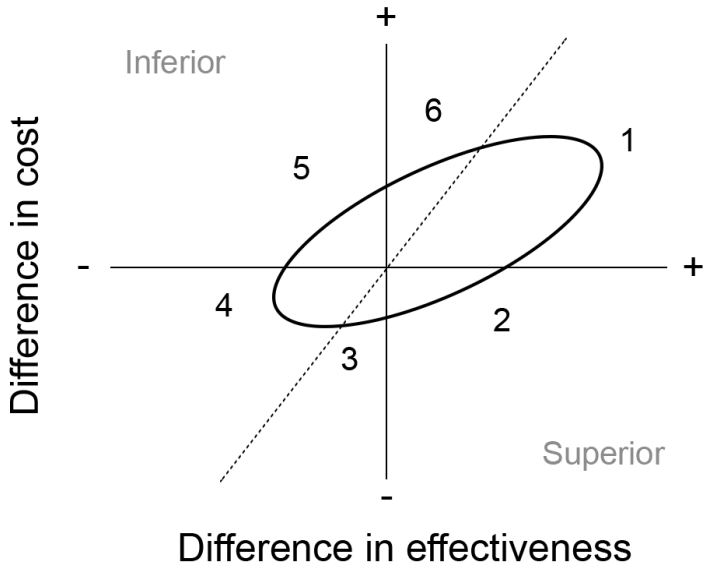

Figure 3. Example of a cost effectiveness plane. The ellipse is an example of the joint distribution of cost and effectiveness for the comparison of two alternatives, a so-called cost effectiveness plane. It is build up from the estimated incremental costs and effectiveness obtained from a Monte-Carlo simulation. The diagonal line signifies the willingness-to-pay threshold. The cost effectiveness plane can be divided into six components. Starting at the top right and going clockwise: 1) more costly and more effective; 2) less costly and more effective, thus superior; 3) less costly, but less effective. In these three cases the alternative would be accepted as they all fall below the willingness-to-pay threshold. 4) less costly and less effective; 5) more costly and less effective, thus inferior; 6) more costly and more effective. In the last three cases the alternative is above the willingness-to-pay threshold, and therefore not accepted.

tree analysis. A decision tree describes the possible clinical pathways that a patient may follow.[12] Probabilities for events can be assigned at each branch, as can costs. A limitation of decision trees is that they assume that a patient travels down a clinical pathway instantaneously. In reality costs and benefits will accumulate over time. Furthermore, probabilities for certain outcomes may vary over time or between patients with different characteristics. Markov modeling, which is procedurally closely related to decision tree analysis, overcomes the time limitation.[12] In a Markov model, simulated patients can cycle through various health states over a period of time. Such a cycle may last a year, for example. During that time a patient gathers quality adjusted life time and costs associated with the health state. Additionally, a patient may switch between health states and different amounts of quality adjusted life time and costs are collected. At the end of the Markov process, the accumulated costs and QALYs are summed. This process can be performed for thousands of simulated patients simultaneously in order to obtain average QALYs gained and costs made. Although more realistic than the decision tree

approach, this Markov cohort still assumes that all patients are equal. Furthermore, the cohort approach assumes that probabilities and costs associated with events in the model have been estimated with a high degree of certainty. To deal with heterogeneity of patients and uncertainty of parameter estimates, sensitivity analyses can be performed. A powerful form of sensitivity analysis is the Monte-Carlo simulation.[11] Instead of cycling an entire cohort of patients through the Markov model at once, patients enter the model one at a time. Costs, utilities and probabilities may be drawn from underlying distributions and tables to reflect both uncertainty and heterogeneity. As a result, estimated costs and possible outcomes more closely reflect the uncertainty of clinical practice.

Cost-effectiveness analyses often require many assumptions. Prudent checking of these assumptions and the (face) validity of the model as a whole is required to assess whether it adequately reflects clinical practice. If modeling assumptions are incorrect, its results may not be useful. Note, however, that if specified correctly, assumptions also underlie the decision for which a model is constructed. Disregarding any model just because it makes many assumptions is flawed reasoning. If anything, a correct, but uncertain model underpins the need for more information.

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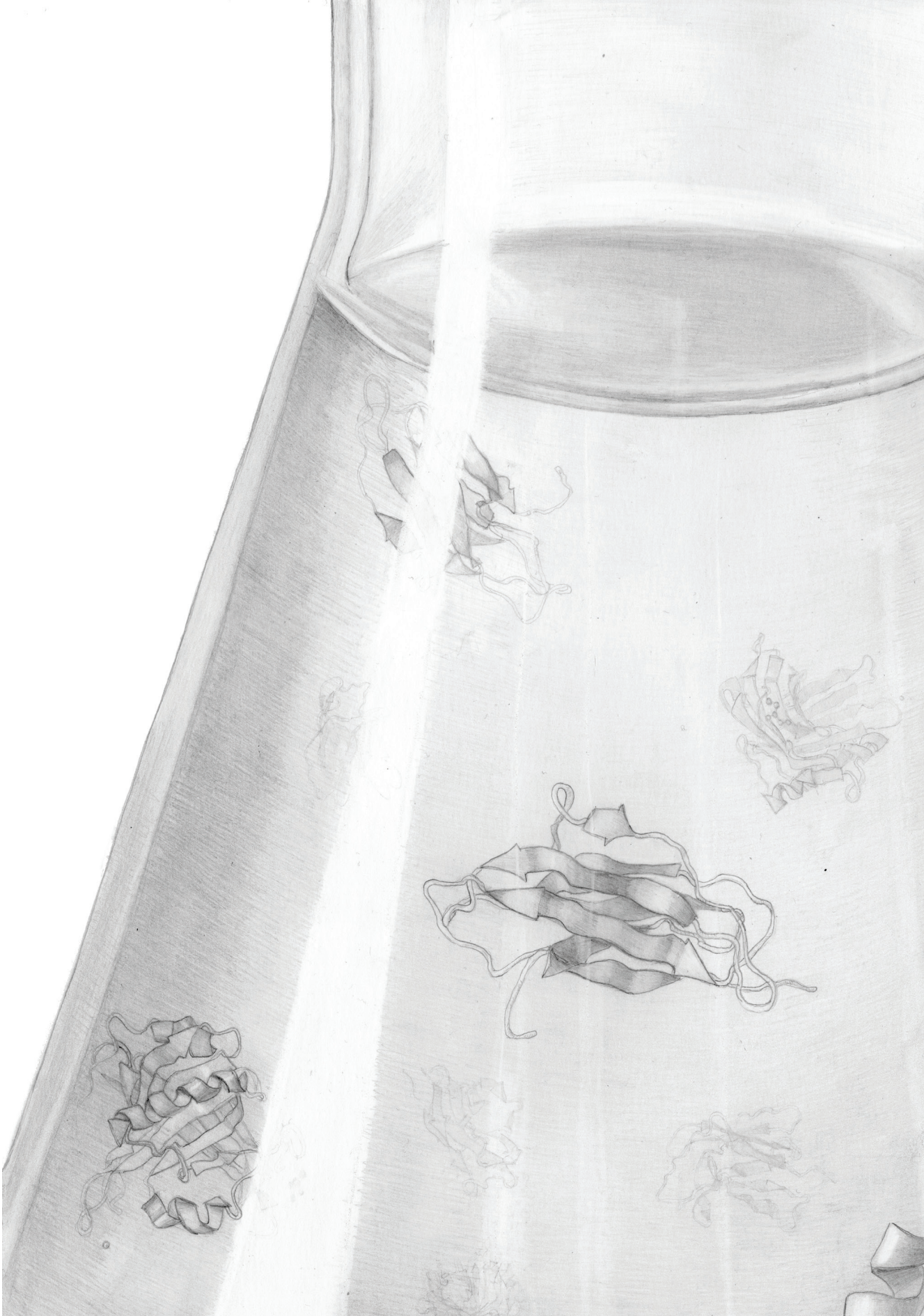

Chapter 3.1: Low Molecular Weight Proteins as Prognostic Markers for Progression in Idiopathic Membranous Nephropathy

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Abstract

Background: Accurate prediction of prognosis in idiopathic membranous nephropathy (iMN) allows restriction of immunosuppressive therapy to patients at high risk for end stage renal disease. Here we re-evaluate urinary low molecular weight proteins as prognostic markers and explore causes of misclassification.
Design, setting, participants, and measurements: In a cohort of 129 patients with serum creatinine concentration <135 μmol/l and proteinuria ≥3.0 g/10 mmol, urinary α_1- ($u\alpha_1m$) and β2-microglobulin ($u\beta_2m$) excretion rate was determined. Urinary α1m and uβ2m/creatinine ratio was also obtained. We defined progression as a rise in serum creatinine ≥50% or ≥25% and an absolute level ≥135 μmol/l.
Results: Median survival time was 25 months and 47% of patients showed progression. The area under the receiver-operating characteristics curve for $u\beta_2m$ was 0.81 (95% CI: 0.73 – 0.89). Using a threshold value of 1.0 μg/min, sensitivity and specificity were 73 and 75%. Similar accuracy was observed for the $u\beta_2m$/creatinine ratio with sensitivity and specificity of 75% and 73% at a threshold of 1.0 mg/10 mmol creatinine. Similar accuracy was found for uα1m and uα1m/creatinine ratio. Blood pressure and cholesterol contributed to misclassification. Repeated measurements improved accuracy in patients with persistent proteinuria, the positive predictive value of $u\beta_2m$ increased from 72% to 89% and the negative predictive value from 76% to 100%.
Conclusions: Urinary excretion of α_1- and β_2-microglobulin predict prognosis in iMN. A spot urine sample can be used instead of a timed sample. A repeated measurement after 6 to 12 months increases prognostic accuracy.

Introduction

Idiopathic membranous nephropathy (iMN) is an important cause of nephrotic syndrome in adults.[1] Spontaneous remission of proteinuria occurs in 30 to 50% of patients.[2,3] Despite treatment with angiotensin converting enzyme inhibitors (ACEi), angiotensin-II receptor blockers (ARB) and statins, between 25 and 50% of patients show progressive loss of renal function.[4,5] Although alkylating drugs improve outcome in patients with iMN,[6-8] these agents often have adverse effects such as bone marrow depression, infections and increased risk of cancer.[8] Therefore one should restrict their use to patients at highest risk of progression to end stage renal disease.

There has been an extensive search for tools that differentiate between patients with a favorable and poor prognosis.[9] Histological markers appeared to be of limited value, whereas the severity of proteinuria is a better marker for outcome.[2-4,10] Remission of proteinuria or increased serum creatinine concentration during follow-up are the most powerful predictors of outcome, however these are late events.[11,12] In past decades, several specific urinary proteins were evaluated as early prognostic markers. Candidates such as TGF-β, βNAG, IgG, complement factors, α_1- and β_2-microglobulin (uα1m and uβ2m) have been proposed.[13-19] In a previous study of 57 patients we showed that uIgG and uβ2m can accurately predict prognosis.[20] Since conservative treatment and prognosis may have changed in recent years, we re-evaluated the data.

Here we report the value of uα1m, uβ2m and uIgG as predictors of outcome in a cohort of 129 patients with iMN. In addition we evaluated the role of these markers in clinical practice using low molecular weight protein/creatinine ratios. We also analyzed possible causes of misclassification and the value of repeated measurements.

Patients and methods

Population

Patients with biopsy proven iMN who attended our clinic for urinary analysis between January 1995 and June 2009 were assessed for this study. Inclusion criteria were normal renal function, defined as serum creatinine <135 μmol/l (≈ 1.5 mg/dL), proteinuria ≥3.0g/10mmol creatinine, and an interval between biopsy and urinary analysis <3 years. Exclusion criteria were participation in the intervention arm of a immunosuppressive therapy trial,21 follow-up duration <1 year or treatment with immunosuppressive drugs prior to urinary analysis. Follow-up was completed until an end point was reached or until June 2010. Patients were followed at our hospital or by nephrologists in referring centers. Patients were treated with diuretics, given dietary sodium restriction, ACE inhibitors and/or angiotensin II receptor blockers and statins according to existing guidelines. Immunosuppressive therapy was advised only in patients with deteriorating kidney function or severe untreatable nephrotic syndrome. Patients with persistent proteinuria were invited for a repeated evaluation after 6 to 12 months.

Data collection

Details of our protocol for the evaluation of patients with iMN are described elsewhere.[20] Patients were instructed to fast overnight and take sodium bicarbonate on the evening before urinary analysis in order to alkalinize urine, because β_2m disintegrates in acidic urine. They did not take diuretics on the morning of urinary analysis. Timed urine samples were collected and blood samples were taken. IgG and uα1m were measured using a BNII nephelometer (Behring, Marburg, Germany) and uβ2m was measured using ELISA.[22] The excretion of total protein and low molecular weight proteins was standardized against urinary creatinine concentration, to obtain a urine protein-creatinine ratio. Data on serum creatinine concentration, urinary protein and creatinine excretion during follow up and use of immunosuppressive therapy, ACE inhibitors, ARBs and lipid lowering drugs were gathered from medical records.

Definition of end-points

We defined progression as 1) a rise in serum creatinine >50%, 2) a rise in serum creatinine >25% and an absolute level ≥135 μmol/l or 3) the need for immunosuppressive therapy because of severe nephrotic syndrome as judged by the treating physician.[23] Partial remission of proteinuria was defined by urinary protein excretion <2.0 g/10 mmol creatinine with stable serum creatinine. We also applied the definition of partial remission as suggested by Troyanov et al (proteinuria <3.5 g/day and a reduction of >50% with a stable kidney function).[2] Remission was considered complete when protein excretion was <0.2 g/10 mmol creatinine. Spontaneous remission means it occurred without immunosuppressive therapy.

Statistical analyses

Median values and inter quartile ranges were calculated. Incidence of patient outcomes was plotted using a competing risks method. The area under the receiver-operator characteristic curve (ROC-AUC) was calculated to compare prognostic value of urinary markers. In order to evaluate calibration, quintiles of predicted risk were created for each urinary marker, both for timed excretion and related to urinary creatinine concentration. Subsequently, the observed risk of progression within risk quintiles was obtained and plotted against the mean predicted probability of progression within each quintile. The Hosmer-Lemeshow goodness-of-fit using five groups was calculated to provide an overall measure of calibration.

Subsequently, we determined cut-off values so that false positive and false negative rates would be minimal and the proportion of correctly classified patients maximized and we calculated sensitivity, specificity, positive and negative predictive values. Finally we created a logistic model using a backward stepwise algorithm with exclusion at $p>0.10$ and re-inclusion at $p<0.05$. The model's ROC-AUC was compared to the AUC for either $u\alpha 1m$ or $u\beta_2m$ to evaluate if it added to discriminatory power. Sources of misclassification were explored by tabulation of baseline characteristics by classification and outcome. One way ANOVA or X^2 tests were used to compare the four groups. Classification according to repeated measurements was cross tabulated by outcome to explore the value of repeated measurements.

Results

Population characteristics

Between January 1995 and June 2009 we evaluated 300 patients with biopsy proven iMN. 169 patients met criteria for enrollment (Figure 3.1.1). In 17 patients follow-up was less than 12 months. No follow-up data were available for 23 patients. Thus, 129 patients were available for analysis. Baseline characteristics are presented in Table 3.1.1. The majority of patients was male and middle aged. Median serum creatinine concentration was 88 µmol/l (inter quartile range 76 - 103) and median proteinuria 8.0 g/10 mmol creatinine (IQR 5.6 - 10.7). Urinary excretion of low molecular weight proteins was increased, with median urinary α1- and β2-microglobulin excretion of 41 (reference < 10) and 0.6 (reference < 0.2) µg/min, respectively. Virtually all patients (99%) received ACE inhibitors and/or ARBs during follow-up and the majority (90%) was treated with lipid lowering medication.

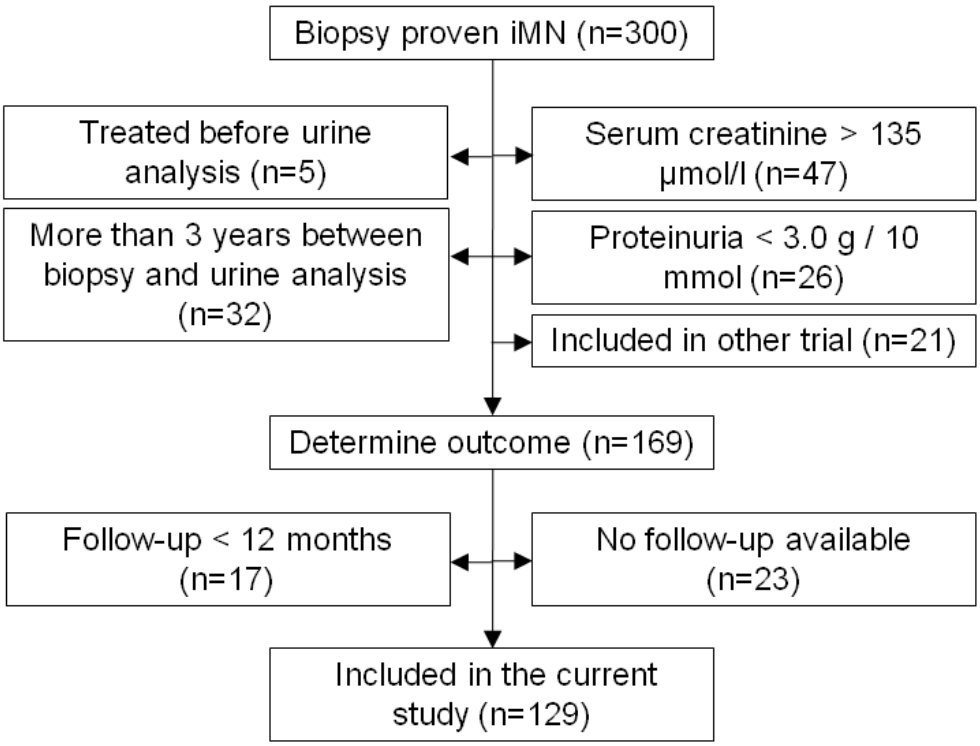

Figure 3.1.1 Flowchart of the inclusion of patients.

Outcomes

Clinical outcome is reported in Table 3.1.1 and illustrated in Figure 3.1.2. Sixty patients (47%) showed progression. In 30 patients serum creatinine concentration increased by >50%, in 24 patients serum creatinine concentration increased >25%

Table 3.1.1. Baseline characteristics of patients with idiopathic membranous nephropathy

Variables	
n (% male)	129 (68%)
age at time of biopsy (years)	51 (43 – 61)
time between biopsy and urine analysis (months)	2 (1 – 4)
Survival time (months)	25 (13 – 51)
MAP (mmHg)	97 (86 – 106)
Laboratory	
serum creatinine (μmol/l)	88 (76 – 103)
serum albumin (g/l)	23 (19 – 28)
serum cholesterol (mmol/l)	7.3 (5.7 – 9.2)
eGFR-MDRD4 (ml/min/1.73m2)	75 (60 – 87)
Urine samples	
proteinuria (g/10 mmol creatinine)	8.0 (5.6 – 10.7)
proteinuria < 4.0 g/10 mmol	9%
proteinuria ≥ 4.0 and < 8.0 g/10 mmol	41%
proteinuria ≥ 8.0 and < 12 g/10 mmol	35%
proteinuria ≥ 12 g/10 mmol	15%
β_2-microglobulin (μg/min)	0.6 (0.2 – 4.8)
α_1-microglobulin (μg/min)	41 (23 – 72)
IgG (mg/24h)	257 (116 – 490)
β_2-microglobulin (mg/10 mmol creatinine)	0.9 (0.3 – 7.0)
α_1-microglobulin (mg/10 mmol creatinine)	36 (57 – 113)
IgG (mg/10 mmol creatinine)	262 (110 – 485)
Selectivity index	0.19 ± 0.09
Medication	
ACEi/ARB use at time of biopsy	22%
ACEi/ARB use during follow-up	99%
Statin use at time of biopsy	13%
Statin use during follow-up	90%
Outcomes	
Progression	47%
50% rise in serum creatinine (n)	30
25% rise and serum creatinine ≥ 135 μmol/l (n)	24
clinical progression (n)	6

Spontaneous remission	47%
partial remission [< 2 g/10 mmol]	61
partial remission [< 3.5 g/10 mmol & 50% reduction]	63
complete remission	26

Data are presented as median (interquartile range). MAP: mean arterial pressure. ACEi: angiotensin converting enzyme inhibitor. ARB: angiotensin-II receptor blocker. eGFR-MDRD4: estimated GFR calculated with the abbreviated MDRD formula.

and reached values ≥ 135 µmol/l, and 6 patients started immunosuppressive therapy because of severe nephrotic syndrome. Of the patients showing progression, 47% did so within 12 months, 72% within 24 months and all within 5 years. In 63 patients proteinuria spontaneously decreased by >50% and reached values <3.5 g/day. With the exception of two cases, proteinuria in these patients decreased to concentrations <2.0 g/10 mmol creatinine. Twenty three percent of patients who developed spontaneous remission (<2.0 g/10 mmol creatinine) did so within 12 months, 59% within 24 months, and 97% within 5 years. Forty three percent of the patients who went into partial remission eventually had a complete remission of proteinuria.

Table 3.1.2. Test characteristics for urinary low molecular weight protein excretion to predict progression in 129 iMN patients.

Threshold value	**sensitivity**	**specificity**	**PPV**	**NPV**	**false positives (n)**	**false negatives (n)**	**test positives (n)**
β2m							
≥ 0.5 µg/min	80%	67%	68%	79%	23	12	71
≥ 1.0 µg/min	73%	75%	72%	76%	17	16	61
≥ 1.5 µg/min	65%	83%	76%	73%	12	21	51
≥ 2.0 µg/min	58%	83%	76%	70%	12	24	47
≥ 2.5 µg/min	55%	84%	75%	68%	11	27	44
α1m							
≥ 40 µg/min	77%	71%	70%	78%	20	14	66
≥ 50 µg/min	65%	83%	76%	73%	12	21	51
≥ 60 µg/min	57%	86%	77%	69%	10	26	44
≥ 70 µg/min	45%	88%	77%	65%	8	33	35
≥ 80 µg/min	42%	90%	78%	64%	11	27	44

PPV: positive predictive value, NPV: negative predictive value, uβ_2m: urinary β_2-microglobulin, ua1m:urinary α_1-microglobulin. Test positives are the number of patients with a urinary α_1- / β_2-microglobulin excretion greater than the threshold value.

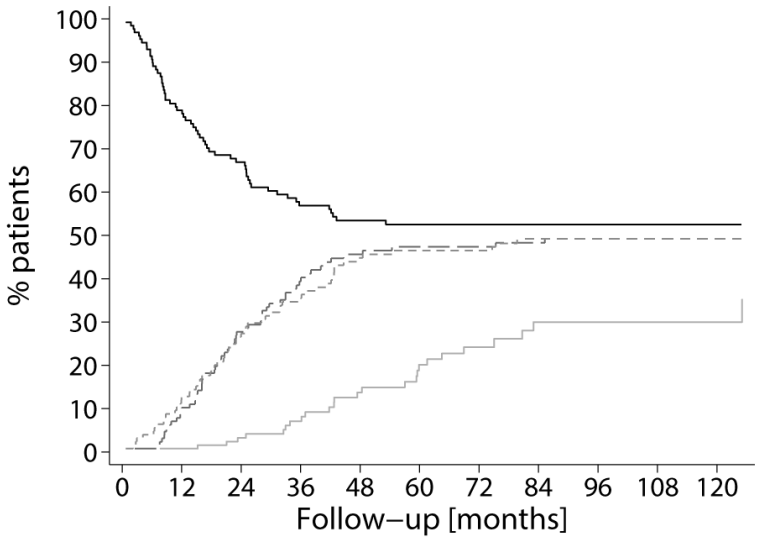

Figure 3.1.2. Patient outcomes. The black line represents renal survival without progression. The grey, short dashed line represents < 3.5 g/day and <50% since baseline, the dark grey, long dashed line partial remission (proteinuria <2.0 g/day), and the light grey line complete remission (proteinuria < 0.2 g/day).

Prognostic value of urinary α1-microglobulin and urinary β2-microglobulin

We plotted a ROC curve for the prognostic accuracy of uα1m, uβ2m and uIgG excretion (Figure 3.1.3, left panel). ROC-AUC was 0.81 (95% confidence interval: 0.73 -0.88) for uα1m, 0.81 (0.73 – 0.89) for uβ_2m and 0.75 (0.66 – 0.84) for uIgG. ROC curves for uα1m and uβ2m and uIgG creatinine ratios are presented in Figure 3, right panel. The ratios yielded similar ROC-AUCs: 0.80 (0.72-0.87), 0.80 (0.72-0.88) and 0.74 (0.66 – 0.83) for uα_1m, uβ_2m and uIgG respectively. The optimal cut off value for the excretion of uβ2m based on our current data is 1.0 μg/min (Table 3.1.2). At this threshold, the positive predictive value (PPV) and negative predictive value (NPV) were 72% and 76%, respectively. For uα1m, a threshold value was determined at 50 μg/min, with a PPV of 76% and NPV of 73%. When excretion was standardized for urinary creatinine concentration, threshold values were 1.0 mg/10 mmol creatinine and 75 mg/10 mmol creatinine for uβ2m and uα1m respectively (supplementary Table S1).

Calibration plots for the low molecular weight protein markers and IgG are shown in figure 3.1.4. The left panels show calibration for timed excretion and the right hand panels show excretion related to urinary creatinine concentration. Table 3.1.3 shows mean predicted by observed risk of progression for the predicted risk quintiles of the prognostic markers. Uβ_2m overestimated observed risk in the lower quintiles of predicted risk, whereas it slightly underestimated risk in the highest quintile. The resulting predicted risk gradient is steeper than the optimal calibration line. The Hosmer-Lemeshow test for IgG does not show an overall

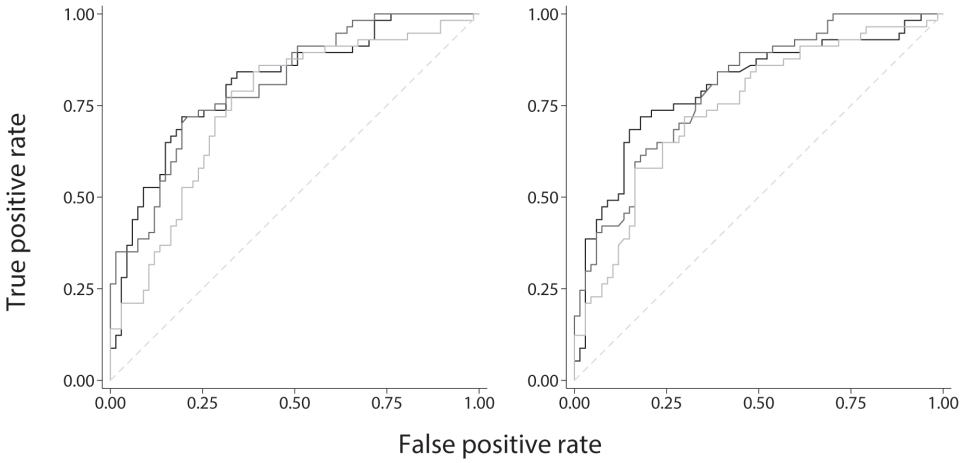

Figure 3.1.3. Left Panel: ROC curves for prognostic accuracy of urinary excretion rate of α_1- (dark grey) and β_2-microglobulin (black) and IgG (light grey). Both α_1- and β_2-microglobulin excretions rates are expressed in µg/min and IgG in mg/24h. Areas under the curve were: uα_1m: 0.81 (0.73 – 0.88), uβ_2m: 0.81 (0.73 – 0.89) and IgG: 0.75 (0.66 – 0.84). Right Panel: ROC curves for the prognostic accuracy of α1- (dark grey) and β2-microglobulin (black) and IgG (light grey). When expressed as mg / 10 mmol creatinine. Areas under the ROC curve were: uα1m/creat: 0.80 (0.72 – 0.87), uβ2m/creat: 0.80 (0.72 – 0.88) and uIgG/creat: 0.74 (0.66 – 0.83).

statistically significant difference between predicted and observed risk using IgG as a prognostic marker. However, IgG does show poor calibration. It underestimates observed risk at the tails of predicted risk, whereas it overestimates risk in the middle quintiles. Finally, α1m appeared to show fairly good calibration across all quintiles of predicted risk.

Sources of misclassification

To evaluate potential sources of misclassification we tabulated baseline characteristics by classification based on uβ2m excretion rate (Table 3.1.4). In general, progressors had higher median serum creatinine (110 and 90 µmol/l versus 80 and 86 µmol/l) and cholesterol concentrations (8.4 and 8.5 versus 6.5 and 6.1 mmol/l) than non-progressors. MAP (94 mmHg versus 93 mmHg) and proteinuria (5.5 versus 6.2 g/10 mmol creatinine) were remarkably similar between misclassified progressors and correctly classified low risk patients, whereas serum albumin levels were markedly higher in non progressing patients whose uβ_2m was <1.0 µg/min than in progressors (27 g/l versus 23 g/l). To further improve prognostic accuracy we created two models, one based on uβ_2m, the other on uα1m. We included baseline MAP, serum cholesterol, serum creatinine, serum albumin and proteinuria. All predictors were log transformed and a stepwise backward selection algorithm was used. The model including uβ_2m also retained serum cholesterol and creatinine as independent predictors. Its ROC-AUC was 0.85 (0.79 – 0.92). A similar model including uα_1m had a ROC-AUC of 0.86 (0.80 – 0.93).

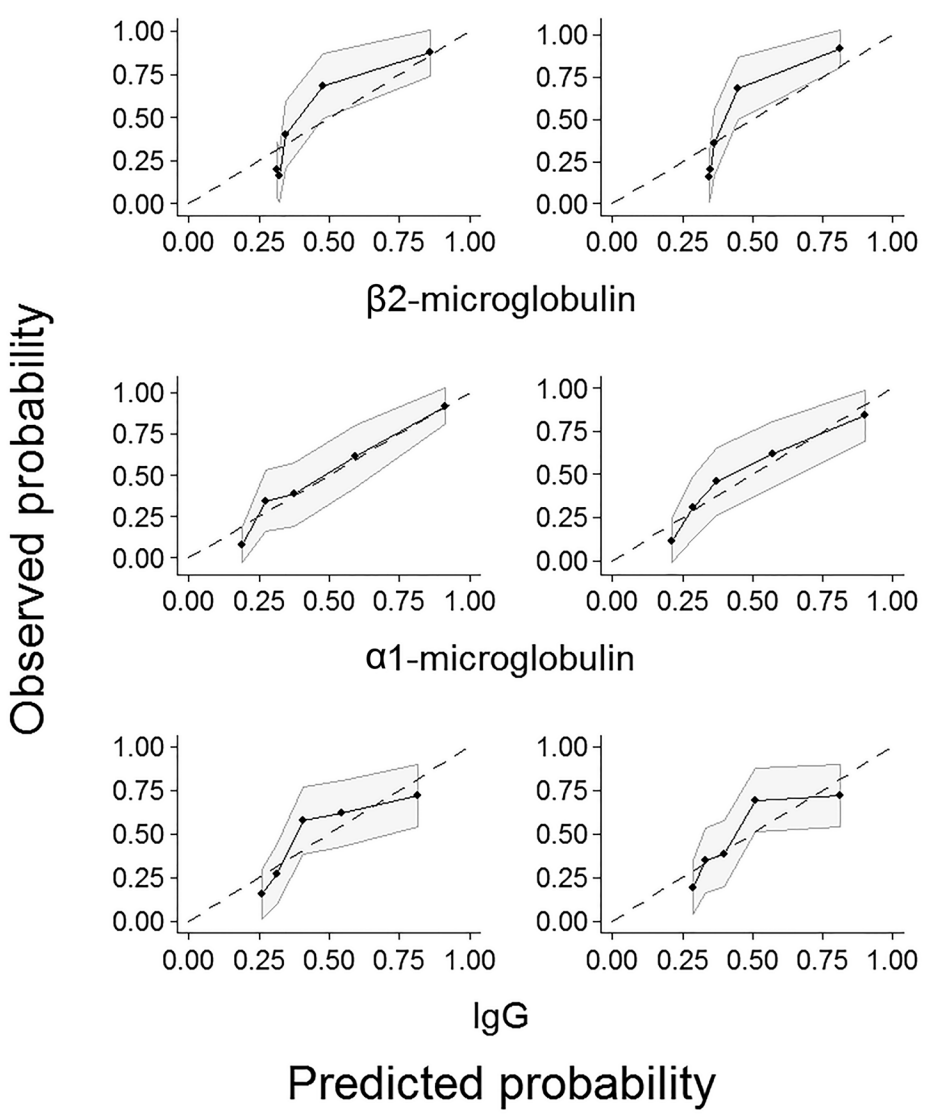

Figure 3.1.4. Calibration plots for progression by urinary markers. The left panels show the results for the timed markers and the right panels for the markers related to urinary creatinine concentration. The dots are the point estimates for mean predicted risk of progression, the shaded area is the 95%confidence interval around the mean predicted risk. The diagonal line is the reference line for perfect calibration

Table 3.1.3. Predicted versus observed probability of progression by quintiles of the predicted probability.

Marker	Risk quintiles	Progression		Hosmer-Lemeshow p
		Predicted	Observer	
uβ2m	I	31%	20%	0.03
µg/min	II	32%	16%	
	III	34%	40%	
	IV	48%	68%	
	V	87%	88%	
uα1m	I	19%	8%	0.42
µg/min	II	27%	35%	
	III	38%	38%	
	IV	59%	62%	
	V	88%	92%	
uIgG	I	26%	15%	0.07
mg/24h	II	32%	27%	
	III	41%	58%	
	IV	54%	62%	
	V	82%	72%	
uβ2m	I	31%	20%	0.004
µg / 10	II	32%	16%	
mmol	III	34%	40%	
creat	IV	48%	68%	
	V	87%	88%	
uα1m	I	19%	8%	0.32
µg / 10	II	27%	35%	
mmol	III	38%	38%	
creat	IV	59%	62%	
	V	88%	92%	
IgG	I	26%	15%	0.11
mg / 10	II	32%	27%	
mmol	III	41%	58%	
creat	IV	54%	62%	
	V	82%	72%	

The Hosmer-Lemeshow goodness-of-fit test gives an overall p-value for the difference between observed and predicted risk over the predicted risk quintiles. Higher p-values indicate better overall calibration. uβ_2m: β_2-microglublin excretion, uα_1m: α_1-microglobulin excretion

The final models are presented in Supplementary Table S2.
We questioned if tubule-interstitial damage could be of value. In 95 patients the interval between kidney biopsy and urine analysis was <3 months. Biopsies of 47 were available for review. Tubulo-interstitial injury (scored 0-3) correlated with $u\beta_2m$ (r=0.58). However the tubule-interstitial injury score did not improve the predictive accuracy in individual patients and did not explain discordances (Supplementary Table S3).

Repeated measurements

We analyzed data of 44 patients with persistent proteinuria who underwent repeated urinary measurements. Baseline characteristics did not differ from total study population characteristics (Supplementary Table S4). At the time of repeated measurements, patients generally had lower blood pressure (MAP 95 versus 89 mmHg) and serum cholesterol values (7.8 versus 6.0 mmol/l) compared to baseline, likely due to intensified conservative treatment. Median serum creatinine concentrations (85 versus 97 µmol/l) and uβ2m (0.5 versus 1.1 µg/min) were higher. We tabulated $u\beta_2m$ at baseline and repeated measurement by outcome in Table 3.5. Patients with a $u\beta_2m$ above 1.0 µg/min at both measurements invariably showed progression (n=11). In contrast, none of the 17 patients with $u\beta_2m$ < 1.0 µg/min at two measurements showed progression. Fifteen (88%) of them went into spontaneous remission. Four patients with $u\beta_2m$ >1.0 at baseline, had $u\beta_2m$ below the threshold at the repeated measurement. Three of them did show progression. In all three patients blood pressure was greatly reduced at the time of the repeated measurement, with a decrease in MAP of 7, 16 and 22 mmHg respectively, leading to very low MAP of 69 and 81 mmHg in two of them. In summary, when $u\beta_2m$ was ≥ 1.0 µg/min in at least 1 of two measurements, PPV for progression was 89% and when $u\beta_2m$ < 1.0 µg/min at both occasions, the NPV was 100%.

Table 3.1.5. Classification according to $u\beta_2m$ excretion at baseline and repeated measurement versus patient outcome in 44 patients with repeated measurements.

Measurement		Outcome (n)	
Baseline	Repeated	Progression	No progression
$u\beta_2m$ ≥ 1.0 µg/min	$u\beta_2m$ ≥ 1.0 µg/min	11	0
$u\beta_2m$ ≥ 1.0 µg/min	$u\beta_2m$ < 1.0 µg/min	3	1
$u\beta_2m$ < 1.0 µg/min	$u\beta_2m$ ≥ 1.0 µg/min	10	2
$u\beta_2m$ < 1.0 µg/min	$u\beta_2m$ < 1.0 µg/min	0	17

The positive predictive value for patients with a least one measurement ≥ 1.0 µg/min was 89%. The negative predictive value for patients with both measurements < 1.0 µg/min was 100%. Of the 11 patients who were classified as progressors and had uβ2m > 1.0 µg/min, 3 had a 50% rise in serum creatinine, 7 had a 25% rise and serum creatinine > 135 µmol/l and 1 patient had severe nephrotic syndrome.

Table 3.1.4. Baseline characteristics for progressors and non-progressors classified

Variables	**Progressors**	
	b2m≥1.0	**b2m<1.0**
n (% male)	44 (75%)	16 (62%)
age at time of biopsy (years)	57 (47 - 64)	49 (44 - 58)
time between biopsy and urine analysis (months)	2 (1 - 4)	2 (0 - 2)
Survival time (months)	11 (6 - 25)	16 (7 - 25)
MAP (mmHg)	100 (89 - 112)	94 (81 - 105)
Laboratory		
serum creatinine (µmol/l)	110 (97 - 119)	90 (68 - 95)
serum albumin (g/l)	20 (17 - 24)	23 (18 - 26)
serum cholesterol (mmol/l)	8.4 (7.0 - 9.8)	8.5 (5.7 - 9.3)
eGFRMDRD4 (ml/min/1.73m2)	58 (53 - 67)	75 (65 - 97)
Urine samples:		
proteinuria (g /10 mmol creatinine)	10.7 (9.3 - 12.7)	5.5 (4.8 - 8.7)
β_2-microglobulin (µg/min)	7.8 (2.3 - 13.8)	0.3 (0.1 - 0.5)
α_1-microglobulin (µg/min)	106 (61 - 131)	31 (20 - 44)
IgG (mg/24h)	511 (356 - 776)	157 (74 - 217)
Selectivity Index	0.27 ± 0.08	0.11 ± 0.05
Medication		
ACEi/ARB use at time of biopsy	36%	20%
ACEi/ARB use during follow-up	100%	100%
Statin use at time of biopsy	20%	7%
Statin use during follow-up	93%	93%
Outcomes		
Progression	100%	100%
50% rise in serum creatinine (n)	18	12
25% rise and serum creatinine >135 µmol/l (n)	24	0
clinical progression (n)	2	4
Spontaneous remission		
partial remission:< 2.0 g/10 mmol	0%	0%
partial remission: <3.5 g/10 mmol and ≥50% reduction	0%	0%
complete remission	0%	0%

Data are presented as mean ± sd or median (interquartile range). MAP: mean arterial receptor blocker. Misclassified progressors are those patients who did show progression but the four groups and X^2 tests to compare medication use and gender.

according to initial excretion of β_2-microglobulin ≥ 1.0 μg/min

Non progressors		p
b2m<1.0	**b2m≥1.0**	
52 (65%)	17 (65%)	0.69
49 (38 - 60)	56 (51 - 64)	0.02
1 (1 - 4)	2 (1 - 4)	0.88
53 (28 - 84)	41 (24 - 54)	
93 (86 - 104)	99 (92 - 104)	0.16
80 (70 - 87)	86 (82 - 91)	<0.001
27 (23 - 31)	22 (17 - 25)	<0.001
6.5 (5.5 - 7.7)	6.1 (5.3 - 7.3)	0.004
85 (78 - 93)	75 (67 - 80)	<0.001
6.2 (4.7 - 8.5)	9.1 (5.9 - 11.0)	<0.001
0.1 (0.2 - 0.4)	2.6 (1.3 - 7.7)	
22 (12 - 37)	50 (39 - 83)	
119 (62 - 219)	351 (158 - 607)	
0.15 ± 0.07	0.21 ± 0.08	<0.001
16%	6%	0.03
98%	100%	0.68
10%	6%	0.26
84%	94%	0.43
0%	0%	
90%	82%	
94%	82%	
40%	29%	

pressure. ACEi: angiotensin converting enzyme inhibitor.ARB: angiotensin-II had uβ2m < 1.0 μg/min. ANOVA was used to compare continuous data between

Discussion

We evaluated urinary excretion of α_1- and β_2-microglobulin as prognostic markers in a cohort of 129 iMN patients with nephrotic range proteinuria and normal serum creatinine concentration. Approximately half of the patients showed progression and the other half went into spontaneous remission. This illustrates that the "rule of thirds" does not apply to iMN patients who present with the nephrotic syndrome and normal kidney function.[24] The majority of patients (61%) reached either disease progression or partial remission within 24 months and 92% within 5 years of follow-up.

Our data indicate agreement between two commonly used definitions of partial remission, i.e. proteinuria <2 g/day versus 3.5 g/day and a decrease >50% from baseline.[2] In our population concordance between the two definitions was almost perfect, only time to remission varied slightly. Patients with high baseline proteinuria tend to achieve remission sooner when the latter definition is used, whereas patients with limited baseline proteinuria have proteinuria less than [2] grams per day before a reduction of 50% is achieved. Thus our data support the use of the definition proposed by Troyanov et al.[2]

We confirmed the prognostic value of uβ_2m. However the AUC was lower than reported in our previous study, 0.81 (95% CI: 0.73 – 0.89) versus 0.94 (0.87 – 1.00).[20] This difference may be caused by a distinction in the definition of endpoints. In our previous study renal death was defined as a rise in serum creatinine ≥50% or an absolute level over 135 μmol/l. In the current study, the second criterion also included a 25% rise in serum creatinine, since an absolute value could lead to biased results.[19] Secondly, we used stricter inclusion criteria in the current study; excluding patients with limited proteinuria. Furthermore, when we inspected baseline characteristics of patients in our current cohort by year of referral, we noted a decline in baseline serum creatinine, albumin and cholesterol, a lower MAP over time and shortened time between biopsy and urine analysis (Supplementary Table S5). Higher referral rates and lower baseline ACEi/ARB use in recent years point toward earlier referrals by participating nephrologists.

Timed urine samples are not routinely taken in all hospitals and uβ2m should be measured after alkalinization of urine by overnight bicarbonate intake. Our current data suggest that a timed measurement of low molecular weight protein excretion may not be necessary. Both α_1m and β_2m related to urinary creatinine concentration had the same prognostic power as the timed excretion. Contrary to uβ2m, uα1m measurement does not require alkalinization and it can be measured using a nephelometric assay, thus spot urine taken at the out-patient clinic for measurement of uα1m/creatinine ratio may be sufficient to predict prognosis.

We attempted to find explanations for the discordance between predicted and actual progressive disease by comparing patient characteristics stratified for prediction and outcome. We observed notable differences in serum cholesterol, creatinine and the ratio between serum albumin and proteinuria. A model that included these variables slightly improved prognostic power. We hypothesize that the higher cholesterol values reflect increased hepatic synthesis and are indicative of higher unmeasured protein losses due to tubular hypermetabolism.

Alternatively, the high cholesterol levels may contribute to progressive renal injury. Although based on a limited number of biopsies, our data suggest evaluation of tubulo-interstitial damage is of no added value.

We evaluated if repeated measurements of uα1m and uβ2m would improve prognostic accuracy. Repeated measurements were done in patients with persistent proteinuria. When one of the measurements was above the uβ2m threshold value of 1.0 μg/min 89% of patients showed progression. Conversely, when both measurements were <1.0 μg/min none of the patients showed progression (NPV=100%). Noteworthy, the data show that changes in blood pressure can influence the results. Low levels of uβ2m and uα1m in the face of very low blood pressure cannot be used with confidence. Alternatively the opposite may also hold true, although we do not have hard data to confirm this.

Compared to α_1m, β_2m shows poor calibration of predicted risk to the observed risk of progression. If low-molecular-weight protein excretion were to be used as continuous or categorical test variables, this would be problematic. In that case α1m would be the preferred marker. However, in the case of a dichotomous test result, the problem of a too steep risk gradient is less pressing. It does not hinder dichotomizing test results, as lower than expected risk in the lowest predicted risk quintiles is inconsequential. Such a result just provides all the more reason not to treat patients with immunosuppression when uβ2m excretion is low. The inverse holds true for high uβ2m excretion.

Our study has several limitations. Our end point to define renal failure can be criticized. However, we feel that it is not justified to delay start of immunosuppressive therapy until doubling of serum creatinine. If we calculate eGFR using the abbreviated MDRD formula 88% of the patients who fulfilled our definition of renal failure had an eGFR value below 60 ml/min/1.73m^2. We performed additional analyses with occurrence of eGFR < 60 ml/min/1.73m^2 as end point. ROC-AUCs for uβ2m and uα1m remained similar and were 0.84 (0.77 – 0.92) and 0.84 (0.76 – 0.92) for uβ_2m in μg/min and mg/10 mmol creatinine respectively. For uα_1m ROC-AUCs were 0.82 (0.74 – 0.89) and 0.82 (0.75 – 0.90) for μg/min and mg/10 mmol respectively. Many patients were referred to our centre for urinary analysis, but followed and treated elsewhere, and we were unable to collect follow up data for all patients. Also, the data we presented on repeated measurements have to be interpreted with some caution as these were performed on a subset of patients with persistent proteinuria. Finally, we were not able to calculate a proteinuria risk score for the cohort, which requires multiple measurements of serum and urine creatinine and proteinuria during each six month period during follow-up.[4] These data were not available.

Conclusions

We have advocated that treatment decisions in the individual patient with iMN must be based on an individualized assessment of risks and benefits.[21] The risks of prolonged nephrotic syndrome should be balanced against those of progression and treatment related complications. Urinary α_1- or β_2-microglobulin measurement can be of value in this balanced decision since they allow an early prediction of prognosis in iMN. A spot urine sample can be used instead of a timed sample. Blood pressure may affect excretion rates. A repeated measurement after six to 12 months increases prognostic accuracy.

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Chapter 3.2: Prognostic value of Risk Score and Urinary Markers in Idiopathic Membranous Nephropathy

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Abstract

Background: Accurate prediction of prognosis may improve management of patients with idiopathic membranous nephropathy (iMN). This study compared the Toronto Risk Score and urinary low-molecular-weight proteins.
Design, setting, participants, and measurements: 104 patients with biopsy-proven iMN who presented between 1995 and 2008 with a well preserved kidney function and nephrotic range proteinuria were included. Urinary β2- (uβ2m) and α1-microglobulin (uα1m) were obtained by timed, standardized measurements and the Toronto Risk Score was calculated using data obtained from medical records. The endpoint was progression, defined as an increase in serum creatinine >50% or >25% with a concentration >135 μmol/l.
Results: 49 patients showed progression. The area under the receiver-operating characteristics (ROC-AUC) curve was 0.78 (95% confidence interval 0.69 to 0.88) for the risk score versus 0.80 (0.71 to 0.89) and 0.79 (0.71 to 0.88) for uβ2m and uα1m, respectively. Differences in ROC-AUC were not significant. Persistent proteinuria did not add accuracy to the Toronto Risk Score. Conversely, its accuracy was not reduced when data from the first six months of follow-up was used. Furthermore, a score based on glomerular filtration rate estimated with the six variable MDRD (eGFR-MDRD6) equation, calculated in the first six months of follow-up, gave a ROC-AUC of 0.83 (0.74 to 0.92), not statistically different from the AUC of the other markers.
Conclusions: The prognostic accuracy of the Toronto Risk Score and urinary low molecular weight proteins were not significantly different. The risk score can be calculated within six months after diagnosis, and a simplified risk score using eGFR-MDRD6 may be sufficient.

Introduction

Idiopathic membranous nephropathy (iMN) is a common cause of adult onset nephrotic syndrome. Untreated, approximately 50% of patients with iMN and nephrotic range proteinuria will develop end stage renal disease.[1] Conversely, almost 50% of patients with nephrotic iMN develop a spontaneous remission of proteinuria. However, it may take anywhere from a few months up to five years to occur.[2] Thus, a delay in treatment would expose the patient to the complications of the nephrotic syndrome such as edema, thrombosis and infections. This dilemma can be tackled through accurate and early prediction of prognosis, as it would allow early treatment and rapid disappearance of the nephrotic syndrome in high risk patients, whilst avoiding unnecessary exposure to toxic therapy in low risk patients.

Almost two decades ago, Pei et al. showed that the magnitude and duration of proteinuria during follow up predicted prognosis better than baseline proteinuria alone.[3] Subsequently, Cattran and colleagues created and validated a risk score for the prediction of progression in iMN which was based on the level of proteinuria during a 6 month period of maximum proteinuria, creatinine clearance at the start of that period and the change in creatinine clearance over the course of those six months.[4] Although accurate, this Toronto Risk Score has some disadvantages. One cannot determine in advance when the period of maximum proteinuria will occur; thus, prolonged observation is necessary. Refraining from therapy prolongs patients' exposition to risks associated with the nephrotic syndrome. In addition, patients are kept in uncertainty. Alternatively, urinary markers have been suggested to predict progression in iMN.[5-9] We showed that urinary excretion of β_2-microglobulin ($u\beta_2m$) or α_1-microglobulin ($u\alpha_1m$) accurately predicted progressive loss of kidney function.[10;11] When re-evaluated, both markers showed somewhat lower sensitivity and specificity than before. This may either be related to changes in patient characteristics at presentation or to improved conservative therapy.[2] Obviously, these factors may also affect prognostic value of the Toronto Risk Score.

Therefore, we compared the prognostic power of the Toronto Risk Score to that of $u\beta_2m$ and $u\alpha_1m$. In addition, we attempted to adapt the Toronto Risk Score to improve its suitability in clinical practice.

Patients and methods

Population

Patients with biopsy-proven iMN who attended our clinic for urinary analysis between January 1995 and June 2009 were screened. As per standard care, potential secondary causes were ruled out by the treating physician, using chest X-ray, serology and routine laboratory investigations as detailed elsewhere.[12] Inclusion criteria were a serum creatinine <135 μmol/l (≈1.5 mg/dL), proteinuria ≥3.0 g/10 mmol creatinine, and time between biopsy and urinary analysis less than one year. Patients with renal insufficiency, who invariably have a worse outcome, and patients with persistent non-nephrotic proteinuria, who almost never progress, were thus excluded. Exclusion criteria were participation in the intervention arm of a therapeutic trial,[13] follow up duration of less than one year, or treatment with immunosuppressive drugs prior to urinary analysis. Follow up data were obtained until an end point was reached or until June 2010. Patients were followed at our hospital or by nephrologists in referring centers. Patients were treated with diuretics, given dietary sodium restriction, ACE inhibitors and/or ARBs and statins according to existing guidelines. Immunosuppressive therapy was advised only in patients with evidence of deteriorating kidney function or severe untreatable nephrotic syndrome. Data of 46 patients were also used in our previous study of low molecular weight proteins.[10]

Data collection and urinary analysis

The study protocol was approved by the RUMC institutional review board and patients provided informed consent. Details of our protocol for the evaluation of patients with iMN were described elsewhere.[10] In summary, patients were instructed to fast overnight; omit the use of diuretics and to take sodium bicarbonate on the evening before and on the morning of the urinary analysis in order to raise urinary pH, as β_2-microglobulin degrades in acidic urine. Timed urine samples were collected and blood samples were taken. Urinary α_1m was measured using a BNII nephelometer (Behring, Marburg, Germany) and uβ_2m was measured using ELISA.[14] Medical records were used to obtain follow-up data on serum creatinine concentration, urinary protein and creatinine excretion and medication use.

Calculation of the Toronto Risk Score

The risk score created by Cattran et al. was calculated as:[4]
Toronto Risk Score = $e^x / (1 + e^x)$; where $x = 1.26 + 0.3 \cdot$ persistent proteinuria $- 0.3 \cdot$ slope creatinine clearance $- 0.05 \cdot$ initial creatinine clearance. Persistent proteinuria, initial creatinine clearance and the slope were calculated during either the first six months of follow up or during the six month period of maximum persistent proteinuria. Creatinine excretion was calculated using the baseline 24 hour urine sample. The total daily urinary excretion of creatinine was assumed to be constant. Glomerular Filtration Rate (GFR) was estimated with the 6 variable MDRD formula,[15] as the MDRD4 equation is not appropriate in persons with

hypoalbuminemia.[16] Persistent proteinuria was assessed using protein creatinine ratios obtained from spot urine samples collected during routine follow up. Since spot and 24 hour proteinuria may differ, we compared the protein creatinine ratio and 24 hours proteinuria at baseline. The sampling methods showed good correlation (r=0.75) and proportional increase (24 hours proteinuria [g/24h] = 0.93 x protein creatinine ratio [g/10 mmol creatinine]. Finally, we chose to limit risk score calculation to the first two years of follow-up, since more than 75% of patients in the study by Cattran et al. had their period of maximum persistent proteinuria during the first two years of follow-up.[4] Moreover, calculations beyond two years of follow up may defeat the purpose of an early marker, as many patients show progression within three years.[2]

Outcome

We defined progression as a rise in serum creatinine of more than 50%, or a rise in serum creatinine of more than 25% and an absolute level higher than 135 μmol/l, or the need for immunosuppressive therapy because of severe nephrotic syndrome as judged by the treating physician.[17] Two of the authors (JH and JvdB) checked all data to establish that the rise in serum creatinine was consistent, persistent and independent of the use of other medication.

Statistical analyses

All analyses were performed using Stata 10.1 (Statacorp LP, TX, USA). Means and standard deviations were calculated for normally distributed variables, and median with interquartile range were used for skewed variables. To obtain an updated prognostic value of the risk score we created receiver-operating characteristic (ROC) curves and calculated the area under the curve (AUC) with 95% confidence intervals (95%CI). The ROC-AUCs were also used to compare the risk score to uβ2m, uα1m and eGFR based risk scores. In order to evaluate calibration, quintiles of predicted risk were created for uβ2m, uα1m and the Toronto Risk Score. Subsequently, the observed risk of progression within risk quintiles was obtained and plotted against the mean predicted probability of progression within each quintile. The Hosmer-Lemeshow goodness-of-fit using five groups was calculated to provide an overall measure of calibration.

Subsequently, we created a logistic regression model containing the individual predictors used in the risk score. Models containing the baseline and change in eGFR-MDRD6 instead of creatinine clearance were created as well. We used these models to calculate the integrative discrimination index (IDI) and relative IDI (rIDI).[18] The IDI quantifies the added discriminatory power of a biomarker to a panel of markers already present in a logistic model, thus it could be used to evaluate the relative contribution of each component of the risk score to the prediction of progression. Finally, we plotted Kaplan-Meier curves by tertiles of the eGFR-MDRD6 risk score to check its calibration with the estimated probability of progression.

Results

Population characteristics

Between January 1995 and June 2009, 300 patients with biopsy-proven iMN were screened. Figure 3.2.1 shows that 163 patients met the inclusion criteria. In total 40 patients were excluded because they had less than 12 months of follow-up or follow-up data were unavailable. Additionally, baseline creatinine clearance could not be determined in two patients, seven patients did not have sufficient data before they reached an endpoint and ten patients did not have sufficient data points within the first 24 months of follow-up. Therefore, our analyses were restricted to 104 patients.

Table 3.2.1 shows the baseline characteristics of our study population. The majority of patients was male (64%), and mean (±SD) age was 52 ± 13 years. In general, patients had well preserved kidney function (endogenous creatinine clearance was 93 ± 31 ml/min/1.73m2) and severe nephrotic syndrome with a mean proteinuria of 8.5 ± 3.3 g/10 mmol creatinine and a serum albumin of 23 ± 6 g/l. Table 3.2.2 shows that conservative treatment was initiated or intensified shortly after biopsy in the majority of patients. Ultimately, all patients used ACE inhibitors or ARBs and 87% used a statin during follow-up. We followed all patients for a median of 4.1 years (inter quartile range [IQR] 2.4 to 6.9). During that time, 47% of the patients showed progression, resulting in a median survival time of 25 months (IQR 12 to 49).

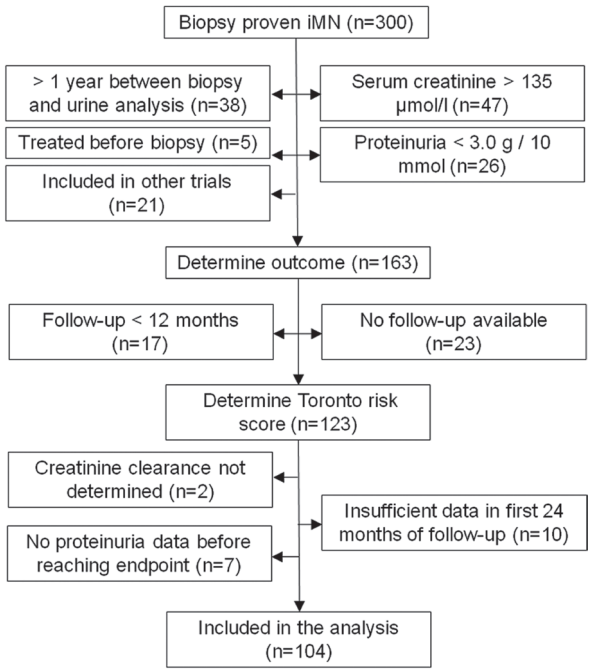


Figure 1. Flowchart for the inclusion of patients with idiopathic membranous nephropathy in the study.

Table 3.2.1. Baseline characteristics of patients with idiopathic membranous nephropathy

Variables	Estimates
n (% male)	104 (64%)
Age at time of biopsy (years)	52 ± 13
Time between biopsy and urine analysis (months)	1 (1 - 3)
Survival time (months)	25 (12 - 49)
MAP (mmHg)	98 ± 16
Laboratory	
serum creatinine (μmol/l)	89 ± 19
serum albumin (g/l)	23 ± 6
serum cholesterol (mmol/l)	7.8 ± 2.5
endogenous creatinine clearance (ml/min/1.73m2)	93 ± 31
Urine samples:	
proteinuria (g/10 mmol creatinine)	8.5 ± 3.3
β_2-microglobulin (μg/min)	0.65 (0.22 - 2.97)
α_1-microglobulin (μg/min)	42 (24 - 76)
β_2-microglobulin (mg/10 mmol creatinine)	0.89 (0.30 - 6.49)
α_1-microglobulin (mg/10 mmol creatinine)	57 (36 - 113)
Progression	47%

Data are presented as mean ± standard deviation, median (interquartile range). MAP: mean arterial pressure. To convert serum creatinine concentration from μmol/l to mg/dl divide by 88.4. To convert mg/10 mmol creatinine to mg/g creatinine divide by 1.13. To convert serum cholesterol values to mg/dl multiply by 38.67.

Comparison of the Toronto Risk Score and urinary low molecular weight proteins

Since intensified supportive treatment influences proteinuria, the magnitude and timing of maximum persistent proteinuria may have changed, and thus the prognostic value of the risk score may have changed as well. Therefore, ROC curves were created to evaluate the prognostic power of the risk score to predict progression. The ROC-AUC was 0.78 (95%CI 0.69 to 0.88), and neither the ROC-AUC for uβ_2m nor that for uα1m differed significantly from the ROC-AUC of the risk score (Figure 3.2.2, Table 3.2.3). The calibration of the Toronto Risk Score and low molecular weight proteins was fairly poor, table 3.2.5, figure 3.2.4. Even though the Toronto Risk Score appeared to show a fair overall calibration, it overestimated progression risk in the first two quintiles and the top predicted risk quintiles. Conversely, it underestimated risk in the third and fourth quintile of the score. Likewise, uβ_2m tended to overestimate risk at lower excretion rates and underestimate risk in the higher quintiles for excretion. As mentioned previously this does not hamper dichotomizing uβ2m test. On the other hand, uα_1m showed the closest overall calibration to observed risk.

Table 3.2.2. Period of maximum persistent proteinuria and initiation of supportive therapy.

Time since urine analysis	Period maximum proteinuria	ACEi/ARB start	Statin start
0-6 months	65%	88%*	61%*
7-12 months	14%	4%	13%
13-18 months	16%	4%	7%
19-24 months	4%	0%	1%
>24 months	§	3%	6%
not started	§	1%	13%

*ACEi, angiotensin converting enzyme inhibitor; ARB angiotensin II receptor blocker; Statin, HMG-CoA-reductase inhibitor; § calculation of the maximum persistent proteinuria was restricted to 24 months; *Start of medication was either before biopsy or during the first six months.*

Prognostic value of the 6 month Toronto Risk Score compared to the Risk Score recalculated during follow-up

Subsequently, analyses were performed in an attempt to improve the original risk score's clinical usefulness. First and foremost, the goal was to determine if recalculating the risk score at later stages of follow up was necessary. A risk score at the start of follow-up could not be calculated for 15 patients, as insufficient data points were available in these first six months. In the remaining 89 patients, the risk score calculated over the first six months was compared to that obtained at the period of maximum persistent proteinuria. The bottom part of table 3.2.3 shows the ROC-AUC for the risk score calculated during the first six months of follow-up, which was 0.76 (95%CI 0.65 to 0.86) and not significantly different from the AUC of the original Toronto Risk Score (p=0.46). In addition, the table shows that the eGFR-MDRD6 risk scores discriminated between stable patients and progressors better than the original, creatinine clearance based risk score. However, the difference was not statistically significant.

Prognostic value of the Toronto Risk Score's individual parameters

The risk score was broken down into its individual parameters in order to investigate which of these contributed most to an accurate prediction of progression. Table 3.2.4 shows the logistic regression coefficients, IDI and rIDI for the original and eGFR–MDRD6 based risk scores calculated at the start of follow-up. The IDI is "the difference in means of predicted probabilities for events and non-events."[18] However, it is unclear what the magnitude of difference means. Therefore, we also calculated the relative IDI, which is the ratio in discrimination slopes for a model with and a model without a marker of interest. In a model which has 4 markers and perfectly predicts outcome, one expects the average contribution from each marker to the discrimination of events and non-events to be roughly 25%. With the introduction of another marker, each of the five markers should contribute 20%. A strong deviation, signifies a respectively high or low relative importance of a marker.

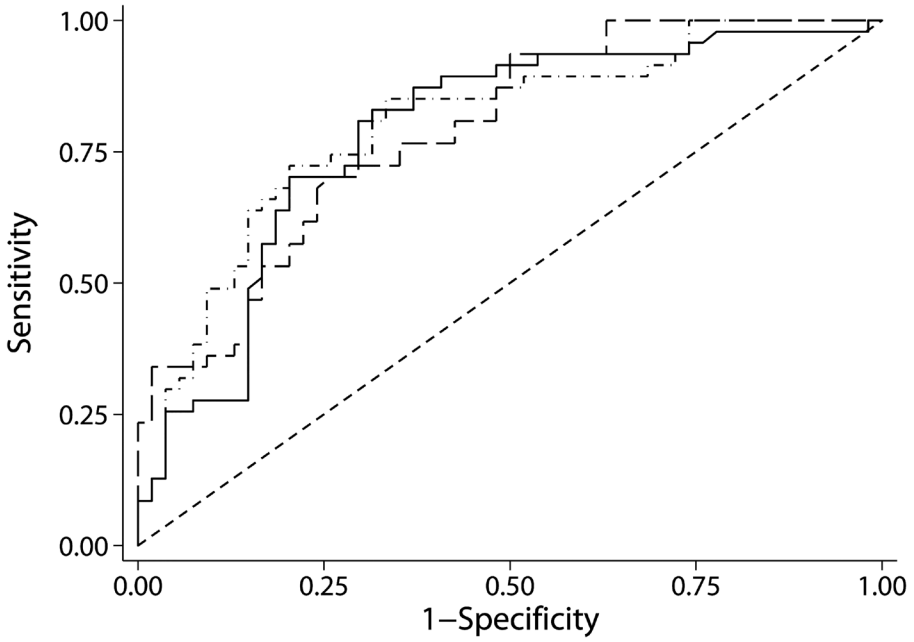
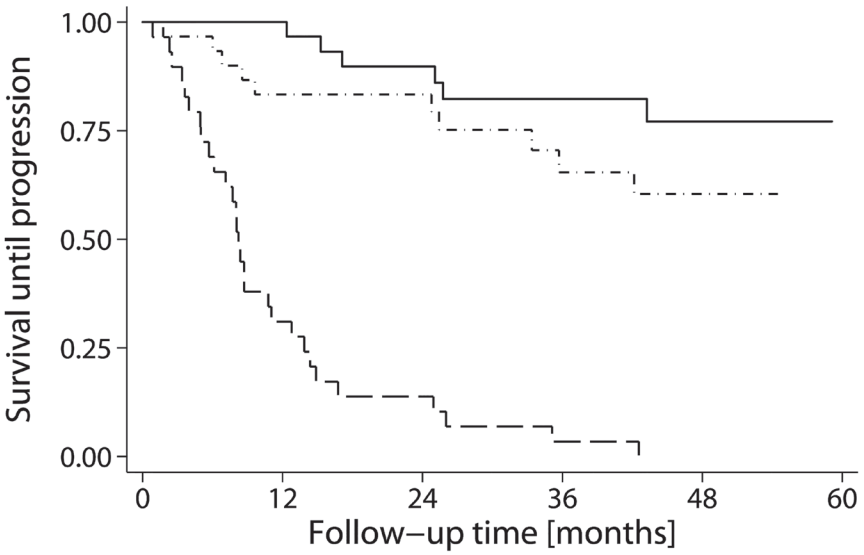


Figure 3.2.2. ROC curves for the Toronto Risk Score (solid), uβ$_2$m (dots and dashes), uα$_1$m (long dashes). The ROC-AUCs for progression were 0.78 (0.69 to 0.88) for the risk score, 0.80 (0.71 to 0.89) for uβ2m, 0.79 (0.71 to 0.88) for ua1m, respectively. None of the areas under the curve differed significantly.

Table 3.2.3. Area under the receiver operating characteristic curves for the Toronto Risk Score and urinary markers to predict progression of idiopathic membranous nephropathy

Marker	**ROC-AUC**	**95% Confidence Interval**			**p**
Toronto Risk Score	0.78	0.69		0.88	ref
uβ$_2$m	0.80	0.71	-	0.89	0.84
uα$_1$m	0.79	0.71	-	0.88	0.85
Toronto Risk Score first 6 months	0.76	0.65	-	0.86	0.46
MDRD6 risk score	0.83	0.74	-	0.91	0.25
MDRD6 risk score first 6 months	0.83	0.74	-	0.92	0.37

uβ$_2$m, urinary β$_2$-microglobulin (µg/min); uα$_1$m, urinary α$_1$-microglobulin (µg/min); ref, reference for the analyses. The MDRD6 risk score was calculated as 1.26 - 0.3 · ΔeGFR-MDRD6 – 0.05 · eGFR-MDRD6 at baseline or at the start of the period of maximum persistent proteinuria, respectively.



Number at risk						
low risk	30	30	24	19	14	12
medium risk	30	25	22	13	11	9
high risk	29	9	4	1	0	0

Figure 3. Survival until progression by tertiles of the eGFR-MDRD6 based Toronto Risk Score without proteinuria, calculated during the first 6 months of follow up. The eGFR-MDRD6 risk score was calculated as the logistic function of 1.26 – 0.3 · ΔeGFR-MDRD6 – 0.05 · eGFR-MDRD6 at baseline. For the Kaplan-Meier plot follow-up duration has been truncated at 60 months. The eGFR-MDRD6 Risk Score was < 0.10 for the low risk tertile (solid line), 0.10 to 0.30 for the medium risk tertile (dots and dashes) and >0.30 for the highest risk tertile (long dashes), respectively.

Table 4. Relative contribution of the risk score parameters for the prediction of progression.

Predictor	**ln(OR)**	**IDI**	**rIDI**
eCcreat	-0.02 (-0.04 - -0.005)	0.07 (p=0.01)	10%
ΔeCcreat	-0.52 (-0.81 - -0.23)	0.21 (p<0.001)	35%
persistent proteinuria	0.09 (-0.04 - 0.22)	0.01 (p=0.54)	1%
GFR-MDRD6	-0.06 (-0.09 - -0.03)	0.22 (p<0.001)	39%
ΔGFR-MDRD6	-0.35 (-0.62 - -0.08)	0.11 (p<0.001)	14%
persistent proteinuria	0.05 (-0.09 - 0.18)	-0.01 (p=0.33)	-1%

ln(OR) is the natural logarithm of the odds ratio obtained with a logistic regression model; IDI: Integrative Discrimination Index; rIDI: relative Integrative Discrimination Index. eCcreat: endogenous creatinine clearance; Δ: change in endogenous creatinine clearance or GFR during the first six months of follow-up.

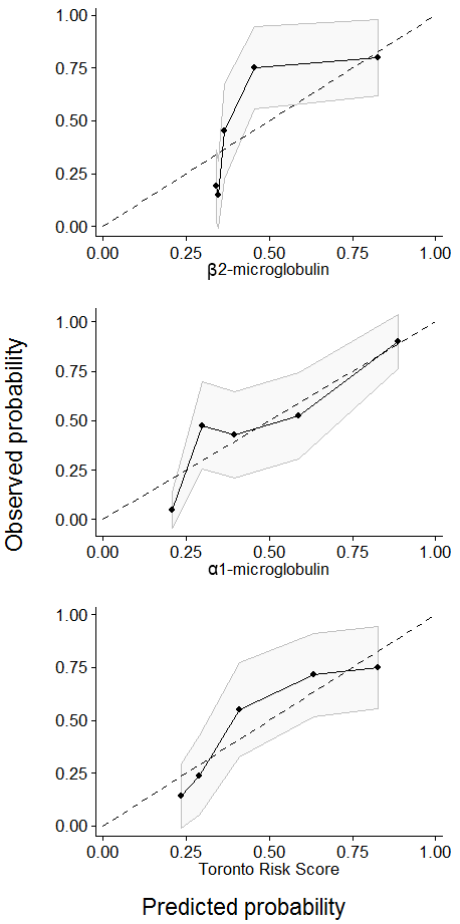


Figure 3.2.4. Calibration plots for uβ_2m (top), uα_1m (middle) and Toronto Risk Score (bottom). The Toronto Risk score calibrates fairly well with the observed risk, as does uα_1m. Urinary β_2m shows a too high predicted risk in low risk patients, and too low in high risk patients.

Persistent proteinuria did not substantially contribute to the prediction of progression.The change in creatinine clearance was more important than baseline creatinine clearance, whereas the opposite was true for the eGFR-MDRD6 based model. Note that the models in Table 3.2.4 are not iterations of the same model with a different number of predictors, therefore the rIDI can only be used to compare predictors within a model and not between models.

Finally, since proteinuria did not contribute to the prediction of prognosis and the eGFR-MDRD6 based risk score appeared to outperform the creatinine based score, we evaluated if the eGFR based risk score without proteinuria could be used to accurately predict prognosis. Figure 3.2.3 shows Kaplan-Meier survival curves by tertiles of the eGFR-MDRD6 based risk score calculated using the first 6 months of follow up. The overall p-value for the logrank test was <0.001. The highest tertile differed significantly from the first, Hazard Ratio (HR)=17.6 (7.0 to 44.3). However, the middle tertile did not differ statistically from the lowest tertile, HR= 2.0 (0.7 to 5.6).

Table 5. Predicted versus observed probability of progression by quintiles of the predicted probability.

Marker	Risk quintiles	Progression		Hosmer-Lemeshow p
		Predicted	Observed	
Toronto	I	23%	14%	0.24
Risk	II	29%	24%	
Score	III	41%	55%	
	IV	63%	71%	
	V	83%	75%	
β_2m	I	34%	19%	0.004
µg/min	II	35%	14%	
	III	37%	45%	
	IV	46%	75%	
	V	83%	80%	
α_1m	I	21%	5%	0.07
µg/min	II	30%	48%	
	III	40%	43%	
	IV	59%	53%	
	V	89%	90%	

The Hosmer-Lemeshow goodness-of-fit test gives an overall p-value for the difference between observed and predicted risk over the predicted risk quintiles. Higher p-values indicate better overall calibration.

Discussion

To the best of our knowledge, the current study is the first to directly compare the two most accurate, validated markers for the prognosis of iMN in patients who present with the nephrotic syndrome. We show that the Toronto Risk Score and urinary α_1- and β_2-microglobulin have similar prognostic value. However, uα1m showed better calibration throughout the measured range. In the low quintiles of β_2m excretion, the risk was markedly lower than the a priori 50% risk of progression. Similarly, the risk in the highest quintiles was 75% and 80%, respectively. Therefore, uβ_2m can be applied as a test dividing patients in low intermediate and high risk categories. In addition, we showed that the risk score can be adapted to improve its clinical applicability. Firstly, accuracy is not higher for risk scores calculated at later stages of follow up, thus a risk assessment can be made after six months of follow up. Secondly, to calculate the risk score, eGFR may be used instead of endogenous creatinine clearance. Moreover, proteinuria did not contribute to the prediction of prognosis. Since serum samples can be readily obtained at out-patient departments, and eGFR-MDRD6 can be automatically reported, our study suggests that a more practical risk score can be used in a clinical setting.

The present study only included patients who presented with the nephrotic syndrome, whereas a considerable proportion of the population (24%) in the Toronto validation study were not nephrotic at presentation.[4] Patients with persistent, limited proteinuria are known to have a favorable prognosis. Perhaps this difference in case mix may explain why proteinuria did not significantly contribute to the prediction of prognosis in our study. Another explanation may be that we used protein-creatinine ratios obtained from spot samples rather than 24 hour proteinuria. However, data from 44 patients who underwent repeated measurements in our previous study show that the variation for protein-creatinine ratio and 24 hour proteinuria is similar at initial and repeated measurement. In addition, the change in protein-creatinine ratio from initial to repeated measurement is strongly correlated (r=0.75) to change in 24 hour proteinuria. Therefore, we concluded that both collection methods are appropriate for prognostic purposes.

In addition, progression was defined as an increase in serum creatinine >50% or >25% with a concentration of at least 135 μmol/l, whereas Cattran et al. used an endogenous creatinine clearance <60 ml/min/1.73m^2 as outcome. However, when analyses were repeated and only those patients who fulfilled both definitions were considered progressors, prognostic accuracy of all markers increased slightly; differences between the Toronto Risk Score and low molecular weight proteins remained the same; recalculating the risk score still did not add prognostic power; and the eGFR-MDRD6 based risk score without proteinuria remained as strong a predictor for prognosis as the original Toronto Risk Score. In conclusion, the choice of end point did not substantially influence our results.

Aggressive use of renin-angiotensin blockers may explain the fact that calculation of the risk score at later stages of follow up does not increase accuracy in the current study. Since these drugs reduce proteinuria, they may influence

occurrence of maximum persistent proteinuria, and thus the prognostic power of the risk score. Indeed, in our cohort the 6 month period of maximum proteinuria fell within the first year of follow-up in 79% of patients. By comparison, in the study by Cattran et al. this was the case in only 53% of patients.[4] Unfortunately, since data on the use of ACE inhibitors and ARBs was not reported, we can only speculate.

We were unable to calculate the risk score for all patients who met the inclusion criteria. These patients were thus excluded. However, the present study population did not differ from the cohort reported previously.[2] Therefore, we consider selection bias due to lack of early follow-up data unlikely.

Finally, although we used the validated multiplication factors from the original paper by Cattran and colleagues, our modifications of the Toronto Risk Score, most notably the use of eGFR-MDRD6 instead of creatinine clearance, have not been externally validated. Therefore, the modified Toronto Risk Score may not perform as well in other patient populations.

Our data show that both urinary low molecular weight proteins and the Toronto Risk Score are suitable to predict prognosis. We prefer the use of urinary markers, since these provide an estimate obtained in a single outpatient visit. Still, if the assay is not available use of the Toronto Risk Score is equally accurate.

It has been suggested that information from the renal biopsy may aid in predicting prognosis. Especially the extent of tubule-interstitial injury has prognostic value.[19] These findings were confirmed in a recent study.[20] However, histology did not independently predict outcome when proteinuria and serum creatinine were taken into account.

In contrast to the original Toronto Risk Score, the eGFR-MDRD6 based risk score does not calculate the predicted probability of progression. Rather, it should be viewed as a dimensionless discriminant score. The higher the score, the more likely progression. The most appropriate way to implement this adaptation of the Toronto Risk Score is to use risk strata. In the present study tertiles of the eGFR-MDRD6 risk based score correspond to discriminant values of <0.10, 0.10 to 0.30, and >0.30, which results in a 20%, 40% and 100% mean five year risk of progression for low, medium and high risk strata, respectively.

Considerable improvements have been made in the prediction of prognosis of iMN patients over the last decades. Proteinuria remains an important predictor. However, when patients present with the nephrotic syndrome, the level of proteinuria does not discriminate between those with a poor prognosis and those with a favorable outcome. Other biomarkers, like those presented in this paper, provide better discrimination in these patients. However, a subset is still misclassified. Therefore, future research should focus on more accurate early markers for disease progression or spontaneous remission, specifically for the patients who are still misclassified with markers presented in this study. Repeated measurements of $u\beta_2m$ or $u\alpha_1m$ may be useful, but more data are still required.[2] A particularly promising marker is the anti-phospholipase A2 receptor antibody level, as it is associated with disease activity in iMN.[21]

Conclusion

Prognosis of iMN patients who present with the nephrotic syndrome can be predicted with either urinary low molecular weight markers or the Toronto Risk Score as these have similar prognostic accuracy. The prognostic power of the risk score is not improved by adding proteinuria. Importantly, a score merely based on the eGFR-MDRD6 equation proved an reasonably accurate predictor. Thus only serum parameters need to be used to predict prognosis of iMN patients, which greatly improves the practical applicability of the Toronto Risk Score. Future studies should focus on the subset of patients who are still misclassified.

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Chapter 4.1: Long Term Outcomes in Idiopathic Membranous Nephropathy using a Restrictive Treatment Strategy

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Abstract

Background: Recently published KDIGO guidelines recommend to limit the use of immunosuppressive drugs in idiopathic membranous nephropathy to patients at the highest risk of kidney failure. However, recommendations are based on natural history rather than direct assessment of a restrictive treatment strategy. We describe the long term outcomes of a large cohort of idiopathic membranous nephropathy patients treated according to such a restrictive treatment policy.
Design, setting, participants, and measurements: We analyzed data of 254 patients who visited our outpatient clinic between 1995 and 2009. All patients were treated with angtiotensin converting enzyme inhibitors and/or angiotensin receptor blockers. Immunosuppressive therapy was recommended in case of deteriorating renal function or untreatable nephrotic syndrome. Primary outcomes for the present study were renal replacement therapy and death. Secondary outcomes included adverse events during follow-up and remission of proteinuria.
Results: In total, 124 patients (49%) received immunosuppressive therapy, which predominantly consisted of cyclophosphamide combined with steroids. Ten year cumulative incidence rates were 3% and 10% for renal replacement therapy and death, respectively. Partial remission rates were 39%, 70% and 83% after one, three and five years. Additionally, one, three and five year complete remission rates were 5%, 24% and 38%. A serious adverse event occurred in 23% of all patients. The most notable complications were infections (17%), leucopenia (18%), cardiovascular events (13%) and malignancies (8%).
Conclusions: In conclusion, the use of a restrictive treatment strategy in idiopathic membranous nephropathy patients yields favorable outcomes, whilst limiting the number of patients exposed to toxic drugs. These results support current KDIGO guidelines.

Introduction

Idiopathic membranous nephropathy (iMN) is the most common cause of adult onset nephrotic syndrome in Caucasians. Recent data show that iMN is an auto-immune disease, with antibodies against PLA2R present in about 70% of patients.[1] The natural course of the disease is variable, with spontaneous remission occurring in 30% to 50% of patients, whereas another 30% to 50% show progressive renal failure.[2,3] In order to avoid progression to end stage kidney disease, patients can be treated with immunosuppressive drugs. Two randomized, controlled trials evaluated the efficacy of the alkylating agents chlorambucil and cyclophosphamide.[4,5] These trials included patients with iMN of recent onset, with normal renal function and nephrotic range proteinuria and showed increased remission rates and improved renal survival in treated patients. However, outcome was favorable in 60% to 65% of the untreated patients. Since most physicians are reluctant to use a treatment schedule which exposes many patients to unneeded, toxic therapy, the use of immunosuppressive therapy in iMN is heavily debated. Accordingly, the recently published KDIGO guidelines recommend to use alkylating agents only in patients at high risk for kidney failure.[6] Thus, in patients with iMN and nephrotic proteinuria the risk of progression to kidney failure should be balanced against the risks and benefits of immunosuppressive therapy.[7] Unfortunately, few studies have evaluated the safety and effectiveness of a restrictive strategy in iMN patients. Studies that were performed included small numbers of patients and had a limited follow-up duration.[8-10]

The present study describes the long term outcome in a large cohort of patients with iMN who were treated according to a restrictive treatment strategy.

Patients and Methods

Patients and treatment

We included adult patients with biopsy proven idiopathic membranous nephropathy who were referred to our clinic between 1995 and 2009. Secondary causes were ruled out per standard policy.[7] Written informed consent was obtained and the study was performed in accordance to the Declaration of Helsinki. Many patients participated in predictor studies described previously and were treated according our restrictive strategy.[2,7] Patients who were not treated restrictively, for instance during a clinical trial on the effects of early treatment, were excluded.[11] A detailed description of the strategy can be found elsewhere.[7] In summary, patients with serum creatinine concentrations less than 135 μmol/l (≈1.5 mg/dL) received supportive treatment. This treatment consisted of blood pressure control and proteinuria reduction with ACEi and/or ARBs, and further blood pressure lowering drugs, if needed, to achieve target levels below 130/80 mmHg. Additionally, statins were given to treat hypercholesterolemia and anti-coagulant therapy was initiated in patients with severe hypoalbuminemia (<20 g/l). In patients with serum creatinine concentrations above 135 μmol/l at presentation or during follow-up, immunosuppressive therapy was advised. Severe, debilitating nephrotic syndrome as judged by the treating physician was considered an indication to start treatment with alkylating agents as well. Oral cyclophosphamide (1.5 mg/kg daily for twelve months) and pulse intravenous methylprednisolone (1 gram on days one to three, 61 to 63 and 121 to 123) in combination with high dose oral prednisone (0.5 mg/kg every other day for five months before tapering) was the preferred immunosuppressive treatment. Occasionally, other drugs were prescribed either as part of a clinical trial or when cyclophosphamide was contra indicated. [11,28] From 1999 onward, trimethoprim-sulfamethoxazole was added to the regimen to prevent pneumocystis jiroveci pneumonia.[11]

Outcomes

Primary outcomes were death, renal replacement therapy, defined as start of chronic dialysis therapy or (pre-emptive) kidney transplantation, and a combination of both. Renal survival was defined as surviving until either renal replacement therapy or mortality, whichever came first. Survival times of patients who did not reach such end-points were censored at the date of the last follow-up visit.

Secondary outcomes were:

1. Pre-specified adverse medical events including those which were either known or suspected to be associated with immunosuppressive therapy, regardless of the therapeutic regimen that the patient received. These pre-specified events were: subfertility (the inability to conceive without medical intervention such as in vitro fertilization), osteonecrosis, hemorrhagic cystitis, malignancies, thrombo-embolic and cardiovascular events (including stroke, deep venous thrombosis and pulmonary embolism, both fatal and non-fatal myocardial infarction and

interventions for peripheral vascular disease).
2. Severe kidney failure, defined as a serum creatinine concentration of 265 μmol/l (≈ 3 mg/dl) or more. This concentration was chosen, because it represents a doubling of serum creatinine from the level at which start of treatment in high risk patients was advised, which was 135 μmol/l (≈ 1.5 mg/dl).
3. Partial remission of proteinuria was defined as a decline in protein : creatinine ratio of at least 50% since biopsy to a level less than 3.5 g/10 mmol creatinine with a stable kidney function.
4. Complete remission of proteinuria was defined as a protein : creatinine ratio of less than 0.2 g/10 mmol creatinine.
5. Relapse of proteinuria was a protein : creatinine ratio greater than 3.5 g/10 mmol creatinine and a 50% increase from the lowest level of proteinuria after remission had occurred.

Data on the primary outcomes and adverse events were obtained from medical records and correspondence. Severe kidney failure, partial remission, complete remission and relapse of proteinuria were determined using laboratory data collected during routine follow-up. Unfortunately, no follow-up laboratory data were available for seven patients. Therefore these patients were only included in the analyses for the primary outcomes.

The follow-up time was calculated from the date of biopsy until outcome or end of follow-up for all analyses, except for relapse of proteinuria. Patients were considered at risk of a relapse only if remission was achieved and until relapse occurred or until end of follow-up. The time from the first remission until a relapse or the end of follow-up was used for the analysis of relapses.

Statistical methods

Baseline data were expressed as proportions, means and standard deviations or medians and interquartile range, where appropriate.

Cumulative incidence rates were calculated using Kaplan-Meier estimates. However, since mortality risk may be competing with renal replacement risk, a competing risks method was used to calculate the cumulative incidence of renal replacement therapy. Similarly, severe kidney failure was deemed competing for the cumulative incidence of both partial and complete remission.

Two sensitivity analyses were performed. First, the main analyses were repeated after excluding patients who initially received immunosuppressive therapy other than cyclophosphamide.

Secondly, in order to assess the potential influence that lacking outcome data may have had on our results, we attempted to obtain vital status for those patients with lacking follow-up data. As a worst case scenario we assumed patients whose status we could not ascertain to be deceased. The date on which we checked vital status was taken as the date of death. Subsequently five and ten year mortality rates were calculated using Kaplan-Meier estimates. Furthermore, we checked if any of the patients whose outcome could not be obtained were registered to have received renal replacement therapy according to the Dutch renal replacement therapy registry (www.renine.nl).

Results

Patient characteristics and treatment

Patient inclusion is shown in Figure 4.1.1. Between 1995 and 2009, 305 patients were evaluated at our centre. Fourteen patients were included in a trial and allocated to an early treatment,[11] and seventeen more were treated prior to referral. Primary outcomes were all cause mortality, renal replacement therapy, being chronic dialysis or transplantation, and a composite of both. Secondary outcomes were: 1) severe kidney failure, defined as a serum creatinine ≥265 μmol/l; 2) a partial remission of proteinuria, defined as proteinuria less than 3.5 g/10 mmol creatinine and a decline of at least 50% from baseline combined with a stable serum creatinine; 3) a complete remission of proteinuria, defined as proteinuria less than 0.2 g/10 mmol; 4) a relapse of proteinuria, defined as the reoccurrence of proteinuria to a level of more than 3.5 g/10 mmol combined with an increase of at least 50% from the lowest level during remission; and 5) adverse medical events during follow-up. Primary outcomes for 20 patients could not be obtained, thus 254 patients were included in the analyses of outcomes and complications.

Table 4.1.1 presents baseline patient characteristics, therapy and outcome data. Most patients were male and mean age was 53 (standard deviation: ±14) years.

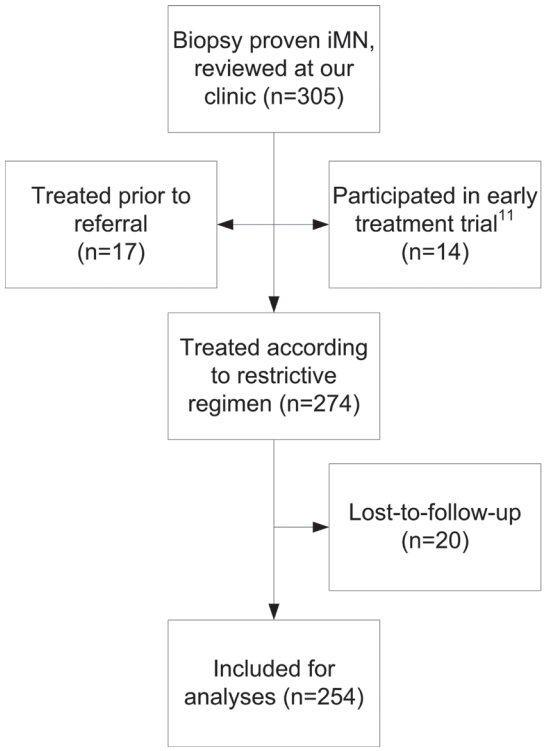

Figure 1. Flowchart for the patient inclusion in the cohort assessing long term outcomes of a restrictive treatment strategy.

The majority of patients (n=226, 89%) had nephrotic syndrome when presenting for urinary analysis, and median estimated Glomerular Filtration Rate (eGFR) according to the four variable Modification of Diet in Renal Disease equation was 71 (inter quartile range: 53 - 85) ml/min/1.73m^2. Patients were followed for a median of 57 months with an interquartile range of 32 to 90 months. At the time of first referral 90% of patients used angiotensin converting enzyme inhibitors (ACEi) and/or angiotensin II receptor blockers (ARBs), and 62% received lipid lowering medication (statins). During follow-up, ACEi/ARB use increased to 96%. In addition, 36% of patients used other blood pressure lowering medication as well. Likewise, statin use increased to 82%, and oral anti-coagulants were prescribed to 36% of all patients. In total 124 patients were treated, 77 because of a serum creatinine greater than 135 µmol/l (1.5 mg/dl). These patients had evidence of renal function deterioration with serum creatinine rising by at least 25% in all, and by >50% in 55 patients. In 47 patients the start of treatment was considered necessary, because of persistent deterioration of eGFR of >5 ml/min per 1.73m^2 per year (n=13), persistent severe hypoalbuminemia (a serum albumin concentration <20 g/l for six months, n= 13), or complications of the nephrotic syndrome (infections and thrombosis, n=6). The rationale for treatment was persistence of the of the nephrotic syndrome itself in the remaining 15 patients. In total, 91 (36%) patients received combined cyclophosphamide/steroid treatment, whereas 33 (13%) received other immunosuppressive drugs, being mycophenolate mofetil (n=19), azathioprine (n=1) or synthetic adrenocorticotrope hormone (ACTH) (n=13). Importantly, 130 patients (51%) received conservative therapy only.

Renal replacement therapy and mortality

The top panel of figure 4.1.2 shows both survival until renal replacement therapy and overall survival. In addition, table 4.1.2 shows cumulative incidence of the primary and secondary outcomes at one, three, five and ten years after first referral. In total, seven patients required renal replacement therapy during follow-up, resulting in a cumulative incidence of 3% (95% confidence interval: 1% to 7%) after ten years. Additionally, the five and ten year mortality rates were 6% (3% to 10%) and 10% (5% to 17%). By comparison, five and ten year mortality rates for the general Dutch population with a mean age of 54 years were 3% and 7%.12 Overall renal survival was 92% (86% to 95%) and 86% (78% to 92%) after five and ten years. In total, 26 patients' serum creatinine concentration reached values over 265 µmol/l, resulting in severe kidney failure incidence rates of 10% (7% to 16%) and 16% (10% to 24%) after five years and ten years. Patients who required immunosuppressive therapy had higher incidences of renal replacement therapy, mortality and severe kidney failure compared to patients who only needed conservative treatment (table 4.1.2).

Remission and relapse of proteinuria

In total, 206 (83%) out of 247 patients (seven patients did not have any follow-up laboratory data) showed a remission of proteinuria. As shown in figure 4.1.2 and table 4.1.2, partial remission rates were 39% (33% to 45%), 70% (64% to 76%) and 83% (77% to 87%) at one, three and five years. Moreover, 97 of the 206 patients with partial remission improved further and attained a complete remission,

Patient characteristics at baseline and start of immunosuppressive therapy

Variables	**Total cohort**	**Conservative treatment**	**Immunosuppressive treatment**	
	Baseline	Baseline	Baseline	Start of therapy
	n=254	n=130	n=124	n=124
% male	68%	61%	76%	
age (years)	53 ± 14	51 ± 14	55 ± 13	56 ± 13
year of biopsy	2003 (1999 – 2006)	2004 (1999 – 2006)	2003 (1998 – 2007)	
follow-up duration (months)	57 (32 – 90)	53 (31 – 82)	59 (37 – 103)	
interval until start of therapy				4 (1 - 13)
BMI (kg/m2)	26.3 (23.8 – 28.8)	26.0 (23.7 – 29.0)	26.5 (23.8 – 28.4)	
eGFR-MDRD4 (ml/min/1.73m^2)	71 (53 – 85)	79 (66 – 89)	59 (42 – 73)	41 (31 - 58)
serum creatinine (µmol/l)	91 (76 – 116)	84 (72 – 94)	109 (86 – 143)	146 (112 - 181)
serum albumin (g/l)	25 (20 – 29)	28 (23 – 31)	21 (17 – 27)	23 (18 - 27)
serum cholesterol (mmol/l)	7.2 ± 2.5	6.6 ± 2.1	7.9 ± 2.6	6.8 (5.7 – 8.4)
protein creatinine ratio (g/10 mmol)	7.1 (4.6 – 10.7)	5.1 (3.2 – 7.7)	10.3 (6.6 – 12.7)	10.4 (7.1 - 15.2)
nephrotic syndrome	89%	82%	97%	
ACEi/ARB use (%)	90%	90%	90%	
statin use (%)	62%	60%	64%	
diuretic use	72%	62%	83%	
other BP lowering medication (%)	24%	15%	33%	
Outcomes (%)				
death	8%	4%	13%	
renal replacement therapy	3%	1%	5%	

serum creatinine > 265 µmol/l	11%	5%	17%
any partial remission during follow-up	81%	79%	84%
any complete remission during follow-up	39%	41%	37%
number of relapses during follow-up			
0	78%	84%	72%
1	19%	14%	23%
≥2	4%	2%	5%

BMI: Body Mass Index, eGFR-MDRD4: estimated glomerular filtration rate according to the four variable Modification of Diet in Renal Disease equation re-expressed for mass spectrometry traceable serum creatinine concentration, ACEi: Angiotensin Converting Enzyme inhibitors, ARB: Angiotensin II Receptor Blockers, BP: blood pressure. Data are presented as mean ± standard deviation, median (inter quartile range) and percentages, respectively

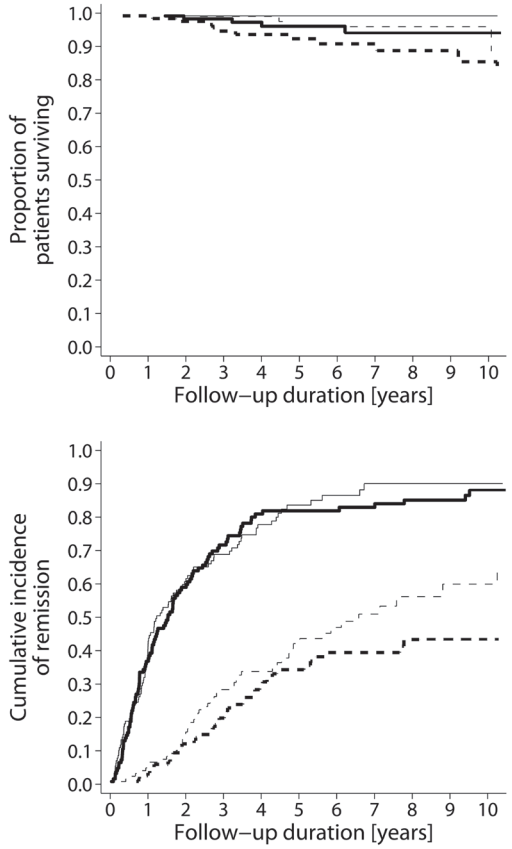

Figure 4.1.2. Outcomes of patients treated with either immunosuppressive and supportive therapy. The thick lines represent patients treated with immunosuppressive drugs, and the thin lines represent patients treated conservatively. The top panel shows survival until renal replacement therapy (solid lines) and mortality (dotted lines). Death was considered competing for renal replacement therapy in the analyses. The bottom panel shows the cumulative incidence of partial (solid lines) and complete remission (dotted lines). Severe kidney failure was considered competing for remission.

resulting in a ten year cumulative incidence of 52% for complete remission. Half of the patients who achieved a remission were treated supportively, 37% were treated with cyclophosphamide and the remaining 13% were treated with other immunosuppressive drugs.

A relapse occurred in 46 of the remitting patients, fifteen of whom had had a complete remission. Thus, cumulative incidences of relapse were 13% (9% to 19%), 19% (14% to 26%) and 27% (20% to 36%) at one, three and five years after remission was first achieved. In total, 25 of the 46 (54%) relapsing patients had re-attained a partial remission of proteinuria at the end of follow-up. Notably, three patients who had had a partial, but no complete remission progressed to renal replacement therapy.

Severe kidney failure

Severe kidney failure, defined as a serum creatinine concentration over 265 μmol/l, was observed in 26 patients. Seven of these patients progressed to end stage kidney failure. We analyzed the course of disease in the other nineteen patients to ascertain whether these patients had progressive disease and thus would be likely to require renal replacement therapy in the near future. Such patients could be considered to have a poor outcome. The clinical details of these nineteen patients and the course of eGFR are illustrated in the table and figures in the supplementary appendix. In summary, five of these nineteen patients were not treated with any immunosuppressive drugs, two of whom refused treatment, and one was not treated due to severe co-morbidity. Furthermore, in nine patients treatment was initiated after eGFR dropped below 30 ml/min/1.73m^2, and in five patients treatment was started when eGFR was still over 30 ml/min/1.73m^2. Inspection of the individual eGFR curves suggested that progressive disease was present in nine of the nineteen patients who did not develop RRT (see supplementary figure s4.1.1), three of whom were treated with cyclophosphamide, two were treated with other immunosuppressive drugs and four were treated conservatively.

KDIGO guidelines recommend that patients with a severely decreased kidney function should not be treated due to the elevated risk of complications and unclear efficacy of immunosuppression in these patients. In total 27 of our patients treated with immunosuppression had an eGFR less than 30 ml/min per 1.73m2 at the start of therapy. Median annual decline eGFR was of 8 ml/min per 1.73m2 since baseline. In 19 patients (70%) eGFR stabilized after the start of therapy to an eGFR decline of less than 1 ml/min per 1.73m2 per year over a median follow-up of 5.1 (IQR 2.6 to 10.8) years from the initiation of treatment onward.

Complications

Overall, 58 patients (23%) suffered a serious adverse event, see Table 4.1.3. Notable complications were infections (n=42, 17%), cardiovascular events (including thrombosis, n=33, 13%)and malignancies (n=20, 8%). As expected serious adverse events were more frequent in patients treated with immunosuppression as compared to those treated conservatively. During follow-up, a malignancy was reported in 20 patients (lung cancer, n=5; hematological malignancy, n=5; gastro-intestinal cancer, n=4; prostate cancer, n=2; bladder cancer, n=2; renal cell carcinoma, n=1; breast cancer, n=1). Malignancies occurred more frequently in cyclophosphamide treated patients.

In total, 21 (8%) patients died, two of whom had required renal replacement therapy during follow-up. Furthermore, eight of the patients who developed a malignancy died during follow-up, five of whom as a result of the cancer. Additionally, four patients died due to cardiovascular causes and a single patient died following an infection. Unfortunately, cause of death could not be ascertained for the other deceased patients.

Table 4.1.2. Incidence of primary and secondary outcomes

Outcome per time period (yrs)	Cumulative incidence for the total cohort (95% CI) (n=254)	Cumulative incidence for the supportive therapy group (95% CI) (n=130)	Cumulative incidence for the immunosupprive therapy group (95% CI) (n=124)
Renal replacement therapy			
1	0% (n / a)	0% (n / a)	0% (n / a)
3	1% (0% - 4%)	1% (0% - 4%)	2% (0% - 6%)
5	3% (1% - 5%)	1% (0% - 4%)	4% (1% - 9%)
10	3% (1% - 7%)	1% (0% - 4%)	6% (2% - 12%)
Mortality			
1	0% (0% - 2%)	0% (n / a)	1% (0% - 4%)
3	3% (1% - 6%)	1% (0% - 5%)	5% (2% - 11%)
5	6% (3% - 10%)	4% (1% - 11%)	8% (4% - 14%)
10	10% (5% - 17%)	4% (1% - 11%)	15% (7% - 25%)
Combined			
1	0% (0% - 3%)	0% (n / a)	1% (0% - 6%)
3	5% (2% - 8%)	2% (0% - 7%)	7% (4% - 14%)
5	8% (5% - 14%)	5% (2% - 13%)	12% (7% - 20%)
10	14% (8% - 22%)	5% (2% - 13%)	20% (12% - 33%)
Serum creatinine >265 µmol/l			
1	3% (2% - 6%)	0% (n / a)	7% (3% - 13%)
3	7% (4% - 11%)	2% (0% - 7%)	12% (7% - 19%)
5	10% (7% - 16%)	4% (2% - 12%)	16% (10% - 25%)
10	16% (10% - 24%)	13% (5% - 31%)	20% (13% - 30%)

Partial Remission									
1	39%	33%	- 45%	41%	32%	- 50%	37%	28%	- 45%
3	70%	64%	- 76%	69%	60%	- 76%	72%	63%	- 79%
5	83%	77%	- 87%	84%	75%	- 90%	82%	74%	- 88%
10	90%	85%	- 94%	90%	81%	- 95%	88%	80%	- 93%
Complete Remission									
1	5%	2%	- 8%	6%	3%	- 11%	3%	1%	- 8%
3	24%	19%	- 30%	28%	20%	- 37%	20%	13%	- 28%
5	38%	31%	- 45%	42%	32%	- 52%	34%	25%	- 44%
10	52%	44%	- 60%	60%	46%	- 72%	45%	34%	- 56%
Relapse									
1	13%	9%	- 19%	12%	7%	- 21%	14%	8%	- 23%
3	19%	14%	- 26%	14%	8%	- 23%	24%	16%	- 35%
5	27%	20%	- 36%	14%	8%	- 23%	39%	28%	- 52%
10	37%	26%	- 50%	38%	19%	- 65%	39%	28%	- 52%

Renal replacement therapy: initiation of chronic hemodialysis or peritoneal dialysis or the receipt of a kidney transplant. The combined primary endpoint is a combination of renal replacement therapy and mortality, whichever came first. Partial remission is defined as a 50% reduction in protein : creatinine ratio to 3.5 g per 10 mmol creatinine or less with a stable serum creatinine. Remission was considered complete when the protein : creatinine ratio was less than 0.2 g/10 mmol. Relapse is the reoccurrence of proteinuria over 3.5 g per 10 mmol creatinine and a 50% increase from the previous known value of proteinuria in a patient with a remission.

Sensitivity analyses

When patients who received immunosuppressive therapy other than cyclophosphamide were excluded, primary outcome rates as well as partial and complete remission rates remained similar. However, relapse rate was lower at 14%, 20% and 31% after three, five and ten years, indicating that relapses occurred more frequently in patients treated with mycophenolate mofetil and/or ACTH. Secondly, we attempted to obtain data on vital status for the 20 patients who were lost-to-follow-up. Of the 20 patients seven had died, another seven were still alive, and we were unable to ascertain vital status for the final six. Thus, in a worst case scenario the estimated five and ten year mortality rates would be 11% (8% to 17%) and 17% (11% to 25%). In addition, none of these 20 patients were registered to have received renal replacement therapy according to the Dutch renal replacement therapy registry (www.renine.nl; personal communication).

Table 3. Adverse events and complications during follow-up

Adverse event	**Cyclophos-phamide**	**Other immuno-suppression**	**Conservative treatment**
	(n=91)	(n=33)	(n=130)
serious adverse event	35 (38%)	11 (33%)	12 (9%)
resulting in (prolongation of) hospitalization	13 (14%)	3 (9%)	2 (2%)
leucopenia	35 (38%)	8 (24%)	2 (2%)
thrombopenia	7 (8%)	2 (6%)	0
liver enzyme abnormalities	7 (8%)	0	0
hyperglycemia	10 (11%)	5 (15%)	1 (1%)
infection	30 (33%)	11 (33%)	1 (1%)
haematuria / cystitis	1 (1%)	0	0
cardiovascular / thrombotic events	18 (20%)	7 (21%)	8 (6%)
subfertility	0	0	0
osteonecrosis	0	0	0
malignancy	14 (15%)	2 (6%)	4 (3%)

Serious adverse events have been defined according to the ICH/GCP guidelines.

Discussion

Our data provide support for the effectiveness of a restrictive treatment strategy in patients with iMN. With the use of this strategy, half of the patients were not exposed to potentially harmful immunosuppressive drugs. Still, long term outcomes were favorable. Overall renal survival was 86% after ten years. Furthermore, a remission of proteinuria was attained in 83% of all patients. Thus, our data provide evidence for the recommendations in the KDIGO guidelines for the treatment of iMN.

A strength of the study is the standard treatment protocol, which closely resembles the recently published KDIGO guideline.[6] In fact, the strategy reported in the present study was even more restrictive than the guideline, as treatment was not necessarily started "if proteinuria was over 4 grams per day and remained over 50% of baseline value, and did not show progressive decline during supportive therapy over a period of six months." Therefore, our data suggests that a watchful waiting policy can safely be extended beyond six months of follow-up if kidney function is stable and patients do not have severe symptoms from nephrotic syndrome. In addition, clinically relevant long term endpoints were recorded and the duration of follow-up was sufficiently long for these endpoints to be attained. Moreover, safety of the treatment strategy was evaluated. Finally, surrogate endpoints such as remission of proteinuria were also evaluated, thus allowing practitioners to assess effectiveness of the therapeutic regimen in an individual patient at an early stage of follow-up.

Our findings suggest that a substantial number of patients with iMN only need supportive therapy. From this we conclude that any future trials that study immunomodulating therapeutic interventions should be restricted to high risk patients only. Furthermore, this study confirms previous reports that complete remission of proteinuria is a good indicator of a favorable prognosis.[13] In addition, patients who showed severe kidney failure were more likely to progress to renal replacement therapy. However, even in these patients immunosuppressive therapy may still stabilize and even improve kidney function , although compared to patients with preserved kidney function, efficacy may be reduced.

In the recently published KDIGO guidelines for iMN, a restrictive treatment strategy in patients with iMN is recommended.[6] According to these guidelines, initial therapy should only be started if proteinuria is persistently over 4 grams per day after six months of conservative therapy and does not show a tendency to decline, or if serum creatinine concentration has risen by more than 30%, or in the presence of severe, disabling or life threatening symptoms related to the nephrotic syndrome. Although the decision to start treatment was not based on proteinuria in our cohort, the renal function criterion and severity criteria were comparable to the guidelines. The KDIGO recommendation was based on studies that included a relatively small number of patients with a limited follow-up duration. When compared to previously published cohorts, the present study included substantially more patients, and patients were, on average, followed for a longer period of time.[8-10] Nevertheless, both remission and renal survival rates in the present study were similar or even higher. Additionally, the overall renal survival attained in our

population was similar to survival in the intervention arms of the trials by Ponticelli et al. and Jha et al. [4,5] Therefore, our results add to the notion that treatment with immunosuppressive therapy should be withheld in patients with a well preserved kidney function, despite the (sometimes prolonged) presence of the nephrotic syndrome. [14] Recently , Howman et al. reported the results of a randomized controlled trial that compared chlorambucil and prednisone to ciclosporin monotherapy or conservative treatment.[15] Immunosuppressive therapy was initiated in patients with established deterioration of renal function. Chlorambucil significantly improved renal outcome, thus confirming that late start (or restrictive use) of immunosuppressive therapy is effective. However, since deterioration of kidney function continued in 58% of the chlorambucil treated patients this study may question the overall efficacy of restrictive therapy with chlorambucil. Clearly, outcome in our treated patients was better, and there may be several explanations. Rather than using chlorambucil, we treated patients with cyclophosphamide, which is better tolerated. As a result patients may be more likely to receive an optimally efficacious dose.[16,17] Furthermore, the UK trial provided chlorambucil for three months, whereas our patients were treated with cyclophosphamide for six to twelve months continuously. Finally ,in the UK trial, patients had an average creatinine clearance, calculated with the Cockcroft and Gault formula, of 50 ml/min, whereas the mean creatinine clearance by the Cockcroft and Gault formula was 62 ml/min at the start of treatment in our cohort. In summary, our patients were treated earlier, longer and with a drug which is often better tolerated.

In the present study, 23% of patients suffered a serious adverse event anywhere during follow-up. Approximately half of these events were due to infections and most of these occurred in patients treated with immunosuppressive agents, despite antibiotic prophylaxis.[11] We observed a similar rate of infections and leucopenia compared to other patient populations often treated with cyclophosphamide. Here, the probability of an infection was approximately 20% compared to 15% to 60% in lupus nephritis. 18 Similarly, the chance of leucopenia in our patients was 20% compared to 15 to 50% in lupus nephritis patients. We observed an 13% chance of cardiovascular and thrombotic complications. By comparison, Lionaki et al. observed an 8% venous thrombosis risk in iMN patients.[19] However, their definition of thrombotic events did not include cardiovascular events.

Malignancy is undoubtedly the most feared long term complication of cyclophosphamide. In our current cohort malignancy occurred in 20 patients (8%), and was significantly more frequent in patients treated with cyclophosphamide (n=14, 15%). Our treatment schedule used from 1995 onward, being 1.5 mg/kg for twelve months and resulting in a cumulative dose of 36 to 46 grams, was based on the study by Bruns et al.,[20] as the Ponticelli regimen had not proven its efficacy in high risk patients. With the twelve month course of cyclophosphamide, we were able to report good results in high risk patients who had renal insufficiency.[10] Based on the available literature at the time, we considered a cumulative dose less than 50 grams acceptable.21 Recent data questioned this threshold value and suggested that cumulative doses over 36 grams should be avoided. [22] Our experience and the results presented in the current study confirm this observation. Therefore, we have recently changed our treatment schedule from twelve to six months cyclophosphamide therapy, halving the cumulative dose.[23] Finally, we observed an

overall mortality rate nearly double of that in the general population. However, our data compared favorably to the 6% to 26% five year mortality rate reported in other iMN cohorts.[8,24,25]

A limitation of the present study is its observational nature. No causal inferences about the efficacy of the strategy nor about the efficacy of individual drugs can be made with the current design. Additionally, there is a possibility of selection bias, as most patients were referred and did not present directly. Patients with an evidently poor prognosis or patients whose disease course is expected to be benign may not have been referred. Furthermore, we were unable to collect complete follow-up data for all patients. However, the potential impact of missing data was addressed with a sensitivity analysis. Additionally, one may argue that the favorable ten year outcomes may not adequately reflect survival over longer periods of time, as 26 patients did show severe kidney failure. In a more detailed analysis we observed nine progressive patients among the [19] patients who had severe kidney failure but did not require renal replacement therapy. In a worst case scenario, one would thus expect sixteen cases of RRT instead of the seven we observed, increasing the ten year cumulative incidence to about 8%. Moreover, the results presented here are only valid for our cyclophosphamide based strategy. Inferences on other immunosuppressive regimens should be made with caution. Finally, given that risk prediction for iMN patients has substantially improved throughout the last decade,[2,26,27] and patients who do require immunosuppressive treatment may be better off when treatment is started at an early stage, the strategy described here may be improved. On the other hand, no differences in outcome were observed in a trial comparing early versus late initiation of therapy.[11] In other words, the optimal timing of therapy still needs to be elucidated.

Conclusion

A restrictive therapeutic regimen yields favorable long term outcomes. Additionally, it results in half of the patients not requiring toxic drugs. Short term and long term side effects remain an important issue and risks of adverse effects should be balanced against the potential benefits of treatment. Overall, our study supports the recommendations in the recently published KDIGO guideline.

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Chapter 4.2: Cancer Risk after Cyclophosphamide Treatment in Idiopathic Membranous Nephropathy

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Abstract

Background and objective: Cyclophosphamide treatment improves renal survival in patients with idiopathic membranous nephropathy (iMN). However, use of cyclophosphamide is associated with cancer. We evaluated the incidence of malignancies in patients with iMN and estimated the cancer risk associated with cyclophosphamide use.
Design, setting and participants: Patients who attended our clinic were included prospectively from 1995 onward. A crude incidence ratio (IR) for the occurrence of malignancy was calculated. IRs were subsequently standardized to potential confounders. Latency between cyclophosphamide therapy and the occurrence of cancer was estimated by stratifying for time since the start of treatment. Finally, Poisson regression was used to obtain a multiple adjusted IR, and to investigate the dose response relationship between cyclophosphamide and cancer.
Results: Data were available for 272 patients, who had a mean age of 51 years, and 70% of whom were male. Median follow-up was 6.0 years (inter quartile range 3.6 to 9.5), and 127 patients were treated with cyclophosphamide. Cancer incidence was 21.2 per 1000 person years in treated patients compared to 4.6 per 1000 person years in patients who did not receive cyclophosphamide, resulting in crude and adjusted IRs of 4.6 (95% confidence interval 1.5 to 18.8) and 3.2 (95% confidence interval 1.0 to 9.5), respectively.
Conclusion: Cyclophosphamide therapy in iMN gives a threefold increase in cancer risk. For the average patient this translates into an increase in annual risk from approximately 0.3% to 1.0%. The increased risk of malignancy must be balanced against the improved renal survival.

Introduction

Idiopathic membranous nephropathy (iMN) is a common cause of nephrotic syndrome in adults. Current guidelines advise treatment with steroids and alkylating agents in patients who are at high risk for end stage renal disease (ESRD) or who have severe, persistent nephrosis.[1] However, many physicians and patients are reluctant to use cyclophosphamide, because of the increased risk of cancer after cyclophosphamide therapy in patients with granulomatosis with polyangiitis (formerly Wegener's granulomatosis), rheumatoid arthritis and non-Hodgkin's lymphoma.[2-4] Ascertaining the association between cyclophosphamide therapy and malignancy in iMN is challenging due to concomitant immunosuppressive therapy, relative rarity of malignancies and the fact that membranous nephropathy may occur secondary to cancer.[5,6] As a result, data on cancer risk in cyclophosphamide treated iMN patients is sparse. Obviously, such information is important when balancing risks and benefits of immunotherapy in iMN. Therefore, we studied the incidence of cancer in a cohort of iMN patients and investigated the role of cyclophosphamide as a risk factor.

Patients and methods

A detailed description of patients and methods is given in the supplementary appendices online.

We included adult patients with biopsy proven iMN who visited our outpatient clinic between 1995 and 2009. Patients were either referred by a family doctor in our catchment area or by an allied centre. Secondary causes were ruled out per standard policy.[7] Written informed consent was obtained. The study was conducted in accordance with the declaration of Helsinki and approved by our hospital's review board.

Most patients were treated according to our restrictive regimen, detailed elsewhere.[7] In summary, patients underwent a standardized, timed urine measurement,[8] and received supportive treatment. Immunosuppressive therapy was advised when patients reached a serum creatinine concentration above 1.5 mg/dL or when patients suffered severe or life threatening symptoms of nephrotic syndrome. Oral cyclophosphamide and pulse intravenous methylprednisolone in combination with high dose oral prednisone was the preferred treatment. Occasionally, alternative immunosuppressive drugs were prescribed.[9,10]

The outcome for the present study was incident malignancy, recorded from medical records and including the date of diagnosis. Mortality and the date of the final consultation were recorded as well.

We pre-specified potential confounders, being age at time of biopsy, gender, ever smoking, having a first degree relative with a history of malignancy, chronic kidney disease stage and nephrotic syndrome. Immunosuppressive therapy was considered a possible confounder if it was initiated prior to cyclophosphamide therapy. Immunosuppressive therapy after cyclophosphamide could have acted as an intermediary, and therefore adjustment could result in underestimation of possible malignancy risk. Gender, date of birth and height were recorded during the urinary analysis, whereas biopsy and follow-up laboratory data were obtained from medical records. We recorded family history, smoking, cyclophosphamide exposure (including total cumulative dose) and the use of other immunosuppressive drugs over the entire follow-up duration.

Statistical methods

Baseline data are presented as mean ± standard deviation, median and inter quartile range (IQR) or frequencies and proportions. X2 test was used to evaluate differences in frequencies. The difference in means for normally distributed variables was compared using t-test. Wilcoxon's rank sum test was used to compare medians for skewed variables.

Person time was calculated from the start of therapy until the occurrence of malignancy or the last consultation date in the cyclophosphamide exposed group. For unexposed patients, person time was calculated from biopsy until malignancy or the last consultation. Ideally, one would start measuring person time for controls at the moment that they would have started treatment. To mimic this moment of exposure, the median time between biopsy and initiation of therapy in the cyclophosphamide group was estimated and deducted from the person time for

each control. Controls who had negative person time as a result were excluded from the analyses.

Subsequently, the cumulative incidence of malignancy was calculated, assuming that death was competing with malignancy risk. Incidence rates were calculated and used to estimate the incidence ratio (IR) of malignancy after cyclophosphamide exposure. In order to estimate the latency between cyclophosphamide exposure and cancer, incidence ratios were calculated by two year strata of person time. Standardized incidence ratios were calculated by weighting for the distribution of the confounders in the cyclophosphamide treated group.[11] Multiple imputation by chained equations was used to impute missing data on smoking status, family history and cumulative cyclophosphamide dose.[12] Poisson regression was used to obtain a multiple adjusted IR for the association between cyclophosphamide and malignancy, taking the imputations into account. In addition, the dose-response relation between cumulative cyclophosphamide exposure and the occurrence of malignancy was investigated by creating 20 gram categories of cumulative exposure and including these in an adjusted Poisson regression. For all analyses, 95% confidence intervals (95%CI) around the incidence ratios were calculated.

Membranous nephropathy can be incorrectly classified as idiopathic when it occurs secondary to an undetected malignancy. These patients are unlikely to respond to supportive therapy, and therefore more likely to receive cyclophosphamide. This would inflate the association between cyclophosphamide and cancer. To address this issue, we analyzed the serum samples of all patients with a malignancy for the presence of antibodies against the phospholipase A2 receptor (antiPLA2R) in serum. Samples were obtained at the time of urine analysis and stored at -80°C and analyzed using a immunofluorescence test (Euroimmun AG, Lübeck, Germany).

In sensitivity analyses, the multivariate analysis was repeated including only patients with complete data. Secondly, we excluded patients who had received immunosuppressive drugs other than cyclophosphamide. Finally, cancer incidence was standardized by age and gender to the general population using incidence estimates obtained by the Netherlands Cancer Registry over the past decade.[13]

Results

Between 1995 and 2009, 305 patients with iMN visited our centre. Twenty patients were lost to follow-up. Eleven patients with negative person times after correction for time-not-at-risk and two cyclophosphamide treated patients were excluded (figure 4.2.1).The present study includes 272 patients (table 4.2.1). The majority of patients was male and the mean age was 51 ± 14 years. Most patients presented with nephrotic syndrome (88%) and well preserved kidney function (mean eGFR 68 ± 24 ml/min per 1.73m^2). During follow-up, 127 patients (47%) received cyclophosphamide, 123 (45%) patients did not receive any immunosuppression, whereas 22 patients did receive immunosuppressive therapy, yet were never treated with cyclophosphamide. Cyclophosphamide treated patients were more likely to be male, older, and had more severe proteinuria and a lower eGFR at the initial visit compared to untreated patients. The median time between biopsy and start of cyclophosphamide treatment was 12 months (IQR: 5 to 26).

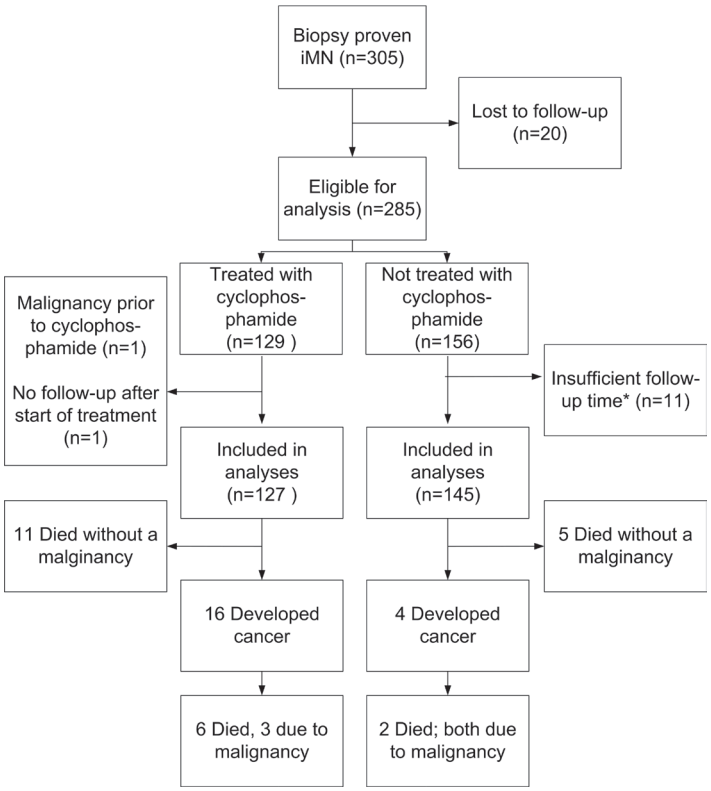

*Figure 4.2.1. Flowchart for inclusion in the cohort. *The median time between biopsy and start of cyclophosphamide exposure was deducted from the exposure time of controls in order to adjust for time not at risk. As a result, eleven patient had negative exposure times and were therefore excluded from the analyses (see statistical methods). Two cyclophosphamide treated patients were excluded, one because of a lung carcinoma in situ prior to treatment and the other was lost to follow-up after the initiation of therapy.*

Table 1: Population characteristics at baseline.

Variables	Cyclophosphamide treated (n=127)	Not treated with cyclophosphamide (n=145)	P
At baseline			
males (n, %)	101 (80%)	89 (61%)	0.001
BMI (kg / m2)	26.6 ± 3.6	27.1 ± 5.0	0.40
age (years)	53 ± 13	49 ± 15	0.05
year of biopsy	2001 ± 7	2002 ± 6	0.10
follow-up duration (years)	7.0 (4.0 – 11.4)	5.4 (3.0 – 8.4)	0.26
positive family history for malignancy*	12/82 (15%)	12/98 (12%)	0.64
current/former smoker*	56/94 (60%)	55/107 (51%)	0.24
eGFR-MDRD4 (ml/min/1.73m2)	60 ± 24	75 ± 22	<0.001
serum creatinine (mg/dl)	1.2 (1.0 – 1.6)	1.0 (0.8 – 1.1)	<0.001
serum albumin (g/dl)	2.2 ± 0.68	2.7 ± 0.63	<0.001
nephrotic syndrome at presentation	121 (95%)	119 (82%)	0.001
protein : creatinine ratio (g / g)	8.9 (5.7 – 11.1)	4.6 (3.0 to 7.6)	<0.001
ACEi/ARB use	112 (88%)	134 (92%)	0.24
statin use	81 (64%)	87 (60%)	0.52
other BP lowering medication	39 (31%)	22 (15%)	0.002
Therapy			
interval from baseline to start of therapy (months)	12 (5 – 26)		
serum creatinine at start of therapy (mg/dl)	1.6 (1.2 – 2.0)		
prior immunosuppressive therapy	27 (21%)	22 (15%)	0.19
cumulative cyclophosphamide dose (g)	37 (21 – 46)	n/a	
Outcomes			
Death	17 (13%)	7 (5%)	0.01
Malignancies	16 (13%)	4 (3%)	0.002

**The denominator differs from the total number of patients due to missing data for this variable. Data are presented as mean ± standard deviation, median (inter quartile range) or percentages where appropriate. BMI: Body mass index; eGFR-MDRD4: estimated glomerular filtration rate, calculated with the abbreviated Modification of Diet in Renal Disease equation for mass spectrometry standardized creatinine; ACEi: angiotensin converting-enzyme inhibitor; ARB: angiotensin II receptor blocker; BP: blood pressure.*

During follow-up (median 6.0, IQR 3.6 to 9.5 years), 20 patients (7%) developed a malignancy and 24 patients (9%) died, five of whom due to a malignancy.
The cumulative incidence of cancer was 11% (95%CI: 7% to 18%) after 10 years (figure 4.2.2). Malignancy incidence was 21.2 per 1000 person years in the cyclophosphamide group compared to 4.6 per 1000 person years in the controls, resulting in an unadjusted incidence ratio of 4.6 (95%CI 1.5 to 18.8). There was no clear relation between incidence ratio and the time of follow-up (figure 4.2.3). Table 2 shows a decrease in the incidence ratio of malignancy after cyclophosphamide exposure when standardized to age, gender and prior immunosuppressive therapy, smoking and positive family history (table 4.2.2).

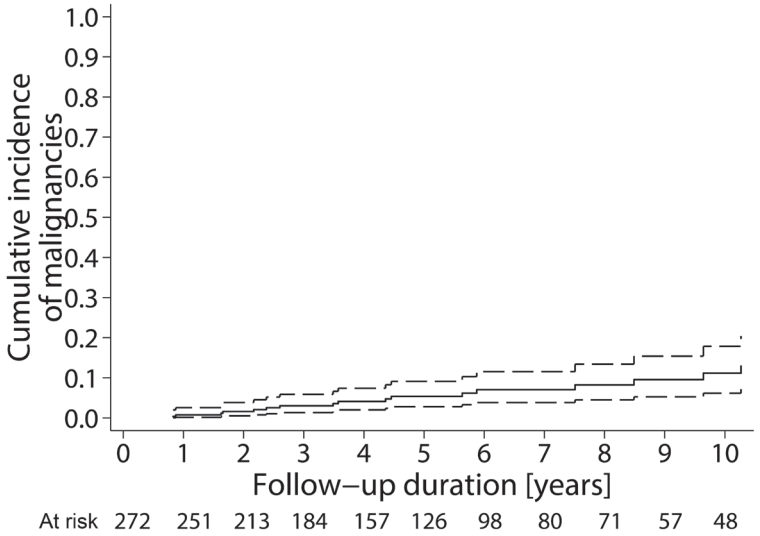

Figure 4.2.2. Cumulative incidence of malignancy for the total cohort of iMN patients, the solid line is the point estimate and the dashed lines are the 95% confidence interval for the cumulative incidence. Death was considered competing for malignancy risk.

Missing values for smoking (n=71, 26%), family history of malignancy (n=92, 34%) and cumulative cyclophosphamide dose (n=15, 6%) were estimated and imputed. After the imputations, the proportion of smokers decreased to 54%, whereas the proportion of patients with a family history of cancer remained 13%. First a multiple adjusted IR of 3.1 (95%CI 0.9 to 9.9) for cyclophosphamide exposure was calculated by entering all possible confounders in a Poisson regression. However, the association between cyclophosphamide treatment and cancer in the Poisson regression was not substantially influenced by smoking status, prior immunosuppressive therapy, family history of malignancy, CKD stage or presence of the nephrotic syndrome. The most parsimonious model included only age and gender as confounding factors and gave an IR for cyclophosphamide exposure of 3.2 (95%CI 1.0 to 9.5).
Figure 4.2.4 shows the adjusted relation between cumulative cyclophosphamide dose in 20 gram categories and malignancy. The respective median cumulative doses per category were 0, 12, 36, 46 and 71 grams. Compared to the untreated patients, the age and gender adjusted incidence ratios by increasing dose category

Table 2: Standardized incidence ratios for malignancy after cyclophosphamide exposure by potentially confounding variables.

Risk factor	Incidence Ratio	95% confidence interval		
Unadjusted	4.6	1.5	-	18.8
Univariate adjusted:				
Age	3.3	1.0	-	10.6
Male gender	3.3	1.1	-	10.0
Smoking	5.8	1.6	-	20.8
Prior therapy	4.2	1.3	-	13.4
Family history of malignancy	7.1	1.6	-	32.0
CKD stage	5.0	1.5	-	18.8
Nephrotic syndrome	4.3	1.3	-	14.1
Age and gender adjusted	3.2	1.0	-	9.5

Age was categorized as <45, 45 to 54, 55 to 64, 65 to 74 and ≥75. The first row shows the unadjusted incidence ratio. The following rows are incidence ratios for cyclophosphamide exposure adjusted for the individual confounder. Ever smokers were compared to patients who never smoked. Prior therapy was defined as immunosuppressive drug therapy other than prednisone before cyclophosphamide therapy was initiated. A multiple adjusted incidence ratio of 3.1 (95%CI 0.9 to 9.9) was obtained with a Poisson regression after multiple imputation of missing values for smoking and family history of malignancy. The most parsimonious model was adjusted only for gender and age, the latter as a continuous variable. The model showed a moderate fit (R2=0.15).

were 4.5 (95%CI 1.1 to 18.4), 2.6 (95%CI 0.7 to 9.4), 3.9 (95%CI 1.0 to 14.7) and 1.7 (95%CI 0.2 to 15.4).

Detailed information on the patients who developed a malignancy is given in table 4.2.3. Three cyclophosphamide treated and two untreated patients died due to a malignancy. Baseline anti-PLA2R was positive in thirteen (65%) patients, negative in five (25%) and unknown in two (10%) patients. There was no association between time to the occurrence of a malignancy and anti-PLA2R serostatus.

In a sensitivity analysis, limited to 201 patients with complete data for smoking and family history of malignancy, the adjusted incidence ratio was slightly higher at 3.3 (95%CI 0.9 to 12.0). In a second sensitivity analysis we excluded all patients treated with other immunosuppressive drugs, therefore 78 cyclophosphamide exposed and 123 unexposed patients remained. The resulting adjusted incidence ratio was 5.1 (95%CI 1.2 to 21.5). When we standardized by age and gender to the general population an incidence ratio of 1.7 (95%CI: 0.9 to 2.6) was observed. Remarkably, the incidence ratio for malignancy in the unexposed patients was 0.5 (95%CI 0.01 to 0.9). Details of the sensitivity analyses are presented in the online appendices.

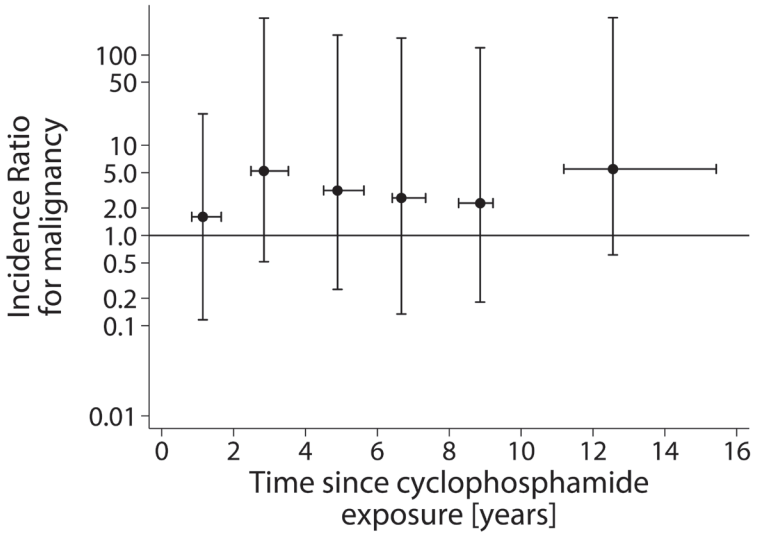

Figure 4.2.3. Unadjusted incidence ratio of malignancy in iMN patients by time since cyclophosphamide exposure in two year strata. Vertical lines represent the 95% confidence intervals around the estimated incidence ratio. The horizontal lines signify inter quartile range of follow-up time within each two year stratum.

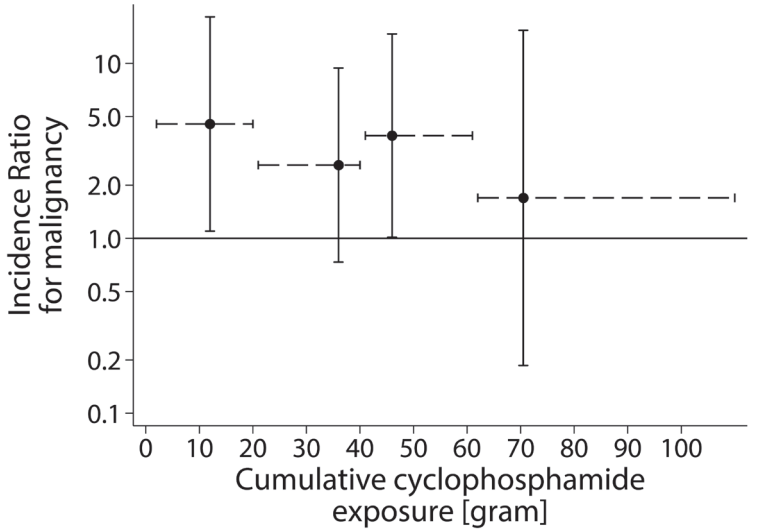

Figure 4.2.4. The dose-response relation between cumulative cyclophosphamide exposure in categories (not treated, 1 to 20, 21 to 40, 41 to 60 and >60 grams) and the adjusted incidence ratio of malignancy in iMN patients. The untreated group has been chosen as the reference, and thus the incidence ratio for malignancy is 1 in that group. The vertical lines represent the 95% confidence intervals around the incidence ratios. The dashed horizontal lines represent the range within the categories for the cumulative cyclophosphamide dose.

Discussion

The present study shows that the incidence of malignancy in cyclophosphamide treated iMN patients was approximately three times higher compared to patients not exposed to cyclophosphamide. For a 55 year old patient this translates into an increase in annual cancer risk from approximately 0.3% to 1.0%.[13] Previous studies have shown that treatment with alkylating agents reduced the incidence of ESRD three to five fold.[14-17] At first glance this decreased renal risk is matched by an increased risk of cancer. However, relative association measures may be misleading. The 10 year ESRD risk of 30% to 40% in untreated patients is best compared to the 10 year malignancy risk of 7% to 18% in treated patients. Moreover, only five of the 20 patients with cancer died as a consequence, and 9% of all patients died during follow-up. By comparison, mortality risk is 50% after only five years in patients with ESRD (Dutch Dialysis and Transplantation Registry: www.renine.nl, personal communication). Moreover, dialysis as well as kidney transplant patients are more likely to die from cardiovascular or infectious causes than malignancy. Admittedly, different types of cancer tend to occur in dialysis and transplant patients.[18] These malignancies may have a different prognosis from the ones observed after cyclophosphamide therapy. Nonetheless, the risks associated with ESRD may still outweigh those of the cyclophosphamide treatment in patients with progressive iMN. Finally, others have shown that very low serum albumin levels as a result of severe nephrosis can result in life threatening thrombo-embolic complications.19 In these patients attenuating the immediate complication risk outweighs the long term malignancy risk.

A strength of the present study is the prospective inception of the cohort, reducing the likelihood of selection bias. Secondly, patients in our cohort have been followed long enough for cancer to occur. In addition, possibly confounding risk factors were taken into account. Moreover, as patients were treated according to a uniform strategy closely adhering to recent guidelines,[20] the data presented here can be generalized to current clinical practice. In addition, sensitivity analyses were performed in which patients treated with other immunosuppression were excluded. In all analyses similar associations between cyclophosphamide use and cancer were observed.

The incidence ratio reported here was similar to that in other patient populations.[2-4,21] Surprisingly, malignancy risk was only 1.7 times higher in cyclophosphamide treated patients compared to age and gender matched persons in the general population. The incidence for unexposed patients was lower than in the general population. A ikely explanation for this finding is that our patients were screened for cancer at baseline. In addition, residual confounding (e.g. due to smoking) may be present when comparing to the general population, whereas confounding was adjusted for in the Poisson regression. Therefore, the results from the regression analysis are most valid.

Remarkably, only two out of twenty observed malignancies were bladder cancers,[22] and no skin cancers were observed. Lefaucheur et al. studied malignancy in iMN patients and did not report skin cancer either.[5] On the other hand, increased risk of skin cancer was observed in patients with rheumatoid arthritis

Table 3. Characteristic for the patients who developed a malignancy during follow-up.

Sex	Cyclophos-phamide treatment	Age at biopsy	Type of malignancy	Died during follow-up	Cause of death	Time between biopsy and malignancy (years)	Time between treatment and malignancy (years)	Time between biopsy and death or final follow-up (years)	Serum anti-PLA2R antibodies at review
Male	No	70	Lung	No		1.9		1.9	n/a
Male	No	37	Lung	Yes	Malignancy	6.9		7.2	-
Male	No	64	Chronic Lymphocytic Leukemia	No		3.4		6.9	+
Male	No	74	Lymphoma n.o.s.	Yes	Malignancy	1.8		1.8	+
Male	Yes	45	Prostate	No		18.9	10.3	18.9	+
Male	Yes	33	Leukemia n.o.s.	Yes	Malignancy	20.6	16.2	20.6	-
Male	Yes	44	Colon	No		12.5	10.9	15.2	+
Male	Yes	70	Prostate	No		12.7	11.8	14.8	n/a
Female	Yes	43	Chronic Myelofibrosis	No		4.5	3.5	10.9	+
Male	Yes	57	Colon	No		10.7	9.6	11.5	-
Male	Yes	66	Prostate	Yes	Unknown	10.1	4.5	14.9	+
Male	Yes	54	Bladder	Yes	Malignancy	8.1	1.6	9.1	+
Male	Yes	48	Chronic Lymphocytic Leukemia	Yes	Cardio-vascular	33.9	8.5	33.9	+

Male	Yes	70	Non Hogdkin Lymphoma	Yes	Unknown	7.6	7.5	11.1	-
Female	Yes	62	Lung	Yes	Malignancy	3.6	3.6	4.6	+
Male	Yes	72	Larynx	No		4.2	2.6	7.8	+
Male	Yes	65	Colon	No		5.8	5.6	7.2	+
Male	Yes	59	Renal Cell Carcinoma	No		8.0	4.4	8.0	+
Female	Yes	66	Acute Myeloid Leukemia	No		2.5	2.2	3.0	+
Male	Yes	79	Bladder	No		1.7	1.7	4.1	-

In two patients serum samples were no longer available, therefore antiPLA2R serostatus could not be ascertained.

and ANCA associated vasculitis.[2,21] However, those patients were treated with multiple drug regimens and for prolonged periods. Perhaps they are more prone to virally induced cancers. Finally, we cannot exclude that small skin cancers were underreported in our study. Conversely, hematologic malignancies were relatively common in our cohort. Therefore, we feel that physicians should maintain awareness for signs of malignancy, including (but not limited to) bladder cancer, in cyclophosphamide treated patients.

We observed some early onset malignancies. We hypothesized that this may be due to inclusion of patients with secondary MN in whom an early diagnosis of cancer was missed. This is unlikely, as the frequency of anti-PLA2R seropositivity in patients with a malignancy was approximately equal to the reported prevalence of anti-PLA2R antibodies in iMN patients.[23] In addition, there was no apparent association between the presence of anti-PLA2R auto-antibodies and the time to malignancy. Several authors have reported the onset of bladder cancer within one year after the start of cyclophosphamide therapy.[24,25] Obviously, reported early cases may reflect incidental findings. Note that our limited data do not allow for further inferences on the association between anti PLA2R antibodies and malignancy risk.

A clear dose response relationship between cyclophosphamide exposure and malignancy risk has been reported in non-Hodgkin's lymphoma.[4] In these patients, risk increased 2.4 fold and 14.5 fold when the cumulative dose was less than 20 and over 50 grams, respectively. Reported cumulative doses in rheumoid arthritis and vasculitis were higher still, averaging over 80 grams in patients who developed cancer.[27] By comparison, the mean cumulative dose in our patients was 37 grams and only 20% of the patients received more than 50 grams. We did not observe a clear dose response relation, possibly due to the uniform treatment that patients received.

When weighing the risks and benefits of cyclophosphamide treatment other complications should be considered. About one in three patients suffers a serious adverse event,[20] being mostly leucopenia, infections and thrombotic complications shortly after the start of therapy. Particularly worrisome for patients of a reproductive age is the risk of infertility. In these patients the duration of cyclophosphamide therapy is limited to three months, which results in a relatively safe cumulative dose of less than 10 grams.[27] Other drugs have been suggested as first line treatment, such as MMF, cyclosporin A, tacrolimus and rituximab.[28,29] Although these drugs induce remission of proteinuria, they have not been unequivocally shown to be as effective as cyclophosphamide in a direct comparison of long term outcome. Moreover, MMF and calcineurin inhibitors have been associated with malignancy in the transplantation setting, possibly via viral mediators.[19] For rituximab on the other hand, data on long term risks are limited.[30] Thus, more data is needed on the efficacy and safety of other immunosuppressive drugs before replacing cyclophosphamide in the treatment of iMN.

When interpreting the data a few issues have to be taken into account. Although screening for malignancy in patients with membranous nephropathy is standard clinical practice, we cannot exclude that malignancies at the time of diagnosis may have been missed. Conversely, there was no standard screening for cancer during follow-up. Because of its known carcinogenic effects, physicians may have

been more proactive in screening patients treated with cyclophosphamide.[2-4] This may have inflated incidence ratios. Although missing values for potential confounders were dealt with in accordance with best practice, incomplete reporting may have led to misclassification of confounder status. Additionally we cannot exclude, residual confounding due to unknown or unrecorded confounders (e.g. alcohol use or environmental exposures). Twenty patients were lost to follow-up shortly after urine analysis. This may have resulted in selection bias of unknown direction. Finally, there were some empty cells in the latency analysis. In order to deal with this in a conservative manner, a single event was added to both the cyclophosphamide and unexposed groups.

Conclusion

We showed that cyclophosphamide therapy gives a threefold increase in risk of cancer within ten to fifteen years after the start of treatment in idiopathic membranous nephropathy. For a 55 year old patient this translates into an increase in annual risk from approximately 0.3% to 1.0%. The data presented here help weighing the benefits against the risks of cyclophosphamide therapy in idiopathic membranous nephropathy, thus enabling physicians and patients to make an informed decision on treatment modality.

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Chapter 5: Cost-Effectiveness of a restrictive treatment strategy in idiopathic membranous nephropathy

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Submitted

Abstract

Background: Treatment with cyclophosphamide improves renal survival in patients with idiopathic membranous nephropathy. However, these drugs can give severe side effects. Thus, KDIGO recommend restricting therapy to high risk patients. However, such a restrictive regimen has not been evaluated experimentally. Likewise, long term outcomes after renal replacement therapy and cost-effectiveness of therapeutic regimens have not been considered previously. In the present study we compared the cost-effectiveness of four alternative treatment strategies for idiopathic membranous nephropathy.

Design, setting and participants: We performed a cost-effectiveness analysis from a healthcare perspective to compare (1) treating all patients with cyclophosphamide, (2) treatment according to the KDIGO guidelines, (3) a restrictive regimen based on a prognostic test with urinary β_2-microglobulin excretion ($u\beta_2m$), and (4) treating all patients conservatively. We created a Markov model for which we obtained transition probabilities, costs and utilities from previous studies and a literature search.

Results: The base case was a 55 year old patient presenting with nephrotic syndrome. The uβ2m based regimen was most cost-effective at a mean cost of €19 963 for an average of 13.94 quality adjusted life years (QALY). Treating all patients with cyclophosphamide, the KDIGO strategy and conservative therapy were more costly and less effective, and therefore considered inferior. Sensitivity analyses showed that treating all patients with cyclophosphamide was cost-effective at higher age and higher discounting rates. The results of a second order sensitivity analysis were similar to the base case analysis.

Conclusion: Restrictive cyclophosphamide therapy based on a prognostic test with urinary β_2-microglobulin was the most cost-effective than treatment for idiopathic membranous nephropathy.

Introduction

The clinical course of idiopathic membranous nephropathy (iMN) is variable. Approximately 30% to 40% of all patients presenting with the nephrotic syndrome progress to end stage renal disease (ESRD) or die prematurely,[1,2] whereas the remainder show spontaneous remission of proteinuria within a few years.[3] Treatment with alkylating agents, most notably cyclophosphamide, is effective in preventing ESRD and mortality.[1,2,4,5] However, alkylating agents may have severe short and long term side effects such as infections and malignancy. Therefore, current KDIGO guidelines recommend that treatment with oral cyclophosphamide should be initiated in patients whose proteinuria persists over 4 grams per day and over 50% of baseline for at least six months without showing signs of decline; or whose serum creatinine has risen by 30% or more within twelve months from the time of diagnosis; or in the presence of severe, disabling or life threatening symptoms of the nephrotic syndrome.[6] However, such a restrictive treatment strategy has not been evaluated experimentally. Moreover, competing restrictive strategies have been proposed. For example, we recently showed that a more restrictive strategy gives similar clinical outcomes whilst limiting the number of patients exposed to potential harmful effects of cyclophosphamide.[7] Additionally, some have argued that the risk of malignancy due to treatment with alkylating agents may outweigh the benefit on renal survival.[8,9] Despite the lack of direct experimental evidence for some of these issues, data from multiple experimental and observational studies can be synthesized to compare long term outcomes for these alternative treatment strategies.

Moreover, resources are sparse in healthcare, either due to a lack of infrastructure in some countries, or due to budgetary concerns in others. Therefore, the optimal treatment strategy needs to be cost-effective as well. Long term outcomes such as mortality, the need for renal replacement therapy and oncologic care are major determinants for healthcare related expenses. Thus, alternative treatment strategies need to be compared on cost-effectiveness beyond the horizon of survival until end stage renal disease. Therefore, we compared the long term cost-effectiveness of four alternative strategies: 1) treating all patients with cyclophosphamide, 2) restrictive treatment according to KDIGO guidelines, 3) deciding appropriate treatment based on a prognostic test, as described previously,[7] 4) and treating patients with conservative therapy only.

Methods

We performed a cost-effectiveness analysis from a healthcare perspective. The target population was adult patients with new onset biopsy proven idiopathic membranous nephropathy and who presented with nephrotic syndrome and well preserved kidney function in an outpatient hospital setting. The base case was a 55 year old patient, as this was the approximate mean age in previous studies.[7] Patients with a decreased kidney function are at high risk for ESRD and should be treated with immunosuppression. On the other hand, patients who do not have nephrotic syndrome are unlikely to show progressive kidney function loss. Therefore, prognostic prediction based on either the KDIGO criteria or urinary β_2-microglobulin ($u\beta_2m$) excretion does not apply to these groups. The study was reported according to available guidelines.[10]

Treatment strategies

We evaluated four treatment strategies, shown in the decision tree in figure 5.1. The first strategy we considered was based on the Ponticelli regimen. We assumed that the immunosuppressive therapy consisted of oral cyclophosphamide (1.5 mg/kg daily for six months) and pulse intravenous methylprednisolone (1 gram on days one to three, 61 to 63 and 121 to 123) in combination with high dose oral prednisone (0.5 mg/kg every other day for five months before tapering). This is our current treatment strategy, and differs somewhat from the original Ponticelli schedule in providing oral cyclophosphamide continuously instead of during alternating months. Secondly, we considered the KDIGO recommendations. Patients were first treated conservatively. However, if partial remission of proteinuria did occur within one year, patients were treated with immunosuppressive therapy. We chose partial remission, as these data were available from a previous study,[11] and we considered a proteinuria of 3.5 g/day sufficiently close to 4 g/day, the recommended level of proteinuria at which treatment should be started. We based the third strategy on a prognostic prediction using uβ2m excretion and subsequent allocation to an immunosuppressive regimen or conservative treatment. If patients had a $u\beta_2m$ greater than 1.0 μg/min, patients were considered test positive. The positive and negative predictive values were 73% and 76%, respectively.[11] In the present simulations, these patients were allocated to immunosuppressive treatment. Test negative patients were allocated to conservative therapy. However, if a patient receiving conservative therapy showed a rise in serum creatinine to a level >135 μmol/l (≈ 1.5 mg/dl), that patient was treated with cyclophosphamide as well.[11] Finally, we included a strategy based on conservative therapy only. Conservative treatment consisted of angiotensin converting enzyme inhibitors and/or angiotensin II receptor blockers in combination with other blood pressure lowering drugs if needed. Statins were used to treat hypercholesterolemia, and platelet aggregation inhibitors were used to prevent thrombosis in patients with deep hypoalbumineamia.

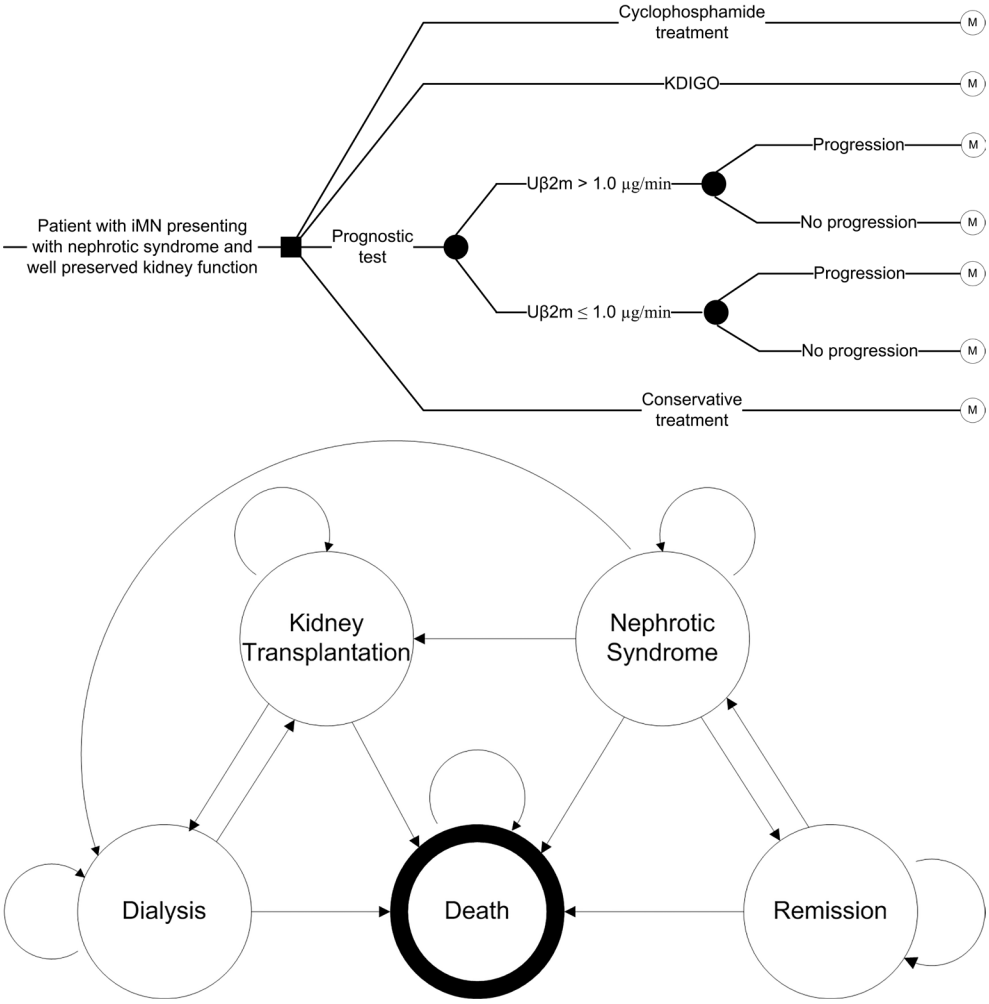

Figure 5.1. Top panel: Decision tree for the cost effectiveness analysis of cyclophosphamide in iMN. Bottom panel: Markov states for the decision model. Complications and/or cancer may occur within a Markov cycle and influence the probability of dying, healthcare related costs and QALYs gained. However, these are not terminal states and are therefore not shown in the Markov model.

The Markov model

We created a decision tree and a Markov process with a one year cycle length. The Markov model ran 10 000 trials. Figure 5.1 shows the possible health states, being death (the absorption state), kidney transplantation, dialysis, remission and nephrotic syndrome. All patients started in the nephrotic state. For simplicity's sake, we considered complications and cancer temporary states which could occur within a cycle. However, we allowed transition probabilities and rewards to vary by age by means of look-up tables, which can be found in the supplementary information.

Table 5.1. Model input: transition probabilities for the Markov process assuming a 55 year patient as base case.

Transitions	Transition probability point estimate	Source
Cancer after dialysis	0.014	Vajdic JAMA 2006[21]
Cancer following cyclophosphamide therapy	0.017	Van den Brand CJASN 2014
Cancer following renal transplantation	0.033	Vajdic JAMA 200621
Cancer without cyclophosphamide (baseline risk)	0.005	Van den Brand CJASN 2014
Complications due to cyclophosphamide therapy	0.047	Van den Brand JASN 2014[7]
Complications due to the nephrotic syndrome	0.013	Lionaki CJASN 2012[22]
Complications while in remission (base risk)	0.0005	Fowkes Eur J Endovasc Surgery 2003[23]
Death after renal transplantation	0.049	Gondos Transplantation 2013[24]
Death due to cancer	0.202	Netherlands Cancer Registry (www.cijfersoverkanker.nl, accessed August 2013)[19]
Death due to cancer after dialysis	0.611	Janus Ann of Oncology 2013[25]
Death due to cancer after NTx	0.389	Apel Clin Transplant 2013[26]
Death due to complications	0.006	Van Gogh Investigators NEJM 2007[27]
Death on dialysis	0.062	Dutch dialysis and transplantation registry (RENINE: www.renine.nl, accessed August 2013 and personal communication)[28]
Death while in remission (base risk)	0.005	Statistics Netherlands (http://statline.cbs.nl, accessed August 2013)[14]

Death while suffering from the nephrotic syndrome	0.013	Controls Ponticelli et al.[1], Jha et al.[2] and Howman et al.[29] Assuming mean age 55 years and 10 year follow-up gives an annual mortality rate of 0.69%. Annual mortality risk in 55 year old Dutch population is 0.63%.14 Thus RR=1.1, assumed 95%CI to be 1.0 to 1.3.
ESRD (when nephrotic syndrome present)	0.042	Ponticelli Kidney Int 1995
Graft loss after kidney transplantation	0.049	Gondos Transplantation 2013,24 the probabilities of graftloss and death are assumed to be approximately equal.
Relapsing nephrotic syndrome	0.048	Van den Brand JASN 2014[7]
Remission following cyclophosphamide therapy	0.619	Van den Brand JASN 2014[7]
Remission when not treated with immunosuppression	0.103	Van den Brand CJASN 2011[11]
Transplantation after dialysis has been initiated	0.318	Dutch dialysis and transplantation registry (RENINE: www.renine.nl, accessed August 2013 and personal communication)[28]

Distributions and tables with age and time dependent transition probabilities used for the probabilistic sensitivity analysis can be found in the supplementary appendix online.

Outcomes we considered were quality adjusted life years (QALYs) and healthcare related costs, and the incremental cost-effectiveness ratio (ICER). The ICER was calculated as the difference in mean cost divided by the difference in mean QALYs. The strategy which was least costly was chosen as the reference for the calculation of ICERs. We deemed a strategy that was more costly and gave fewer QALYs than the reference 'dominated.' If a strategy resulted in higher costs and more QALYs gained, or if a strategy was less costly, but resulted in fewer QALYs gained, we considered it cost-effective if the ICER was greater than the willingness-to-pay threshold.[12] We chose a willingness-to-pay threshold of €80 000 per QALY gained, based on Dutch guidelines.[13] By comparison, willingness to pay thresholds are $62 000 (≈€46 000) is the USA and £23 000 (≈€29 000) in the UK. The lifetime horizon of the Markov process was 30 cycles, similar to the mean life expectancy for 55 year old persons in the Netherlands.[14] All analyses were performed using TreeAge Pro 13 (www.treeage.com).

Data sources and model assumptions

Table 5.1 shows the transition probabilities used in the model and the literature sources from which these probabilities were obtained. We made some assumptions in order to estimate transition probabilities. Most notably, we assumed the risk for malignancy after cyclophosphamide treatment not to be dose dependent within the ranges used in iMN.[15] In addition, we considered thrombo-embolic complications most frequent and important in the nephrotic state. Therefore, we took the rate of deep venous thrombosis in the general population as the comparator risk of complications in the remission state. In addition, leucopenia and infections requiring hospitalization are the most common immediate complications of cyclophosphamide treatment. We added these as a possible one time risk and associated costs in treated patients.

Table 5.2 shows the rewards linked to the different Markov states. We deemed spontaneous remission the preferred state, and therefore set it to reflect nearly perfect health (utility of 0.95 to 1.0). Conversely, we took death as the least preferred state and set its utility to 0. We obtained utilities for nephrotic syndrome and receiving cyclophosphamide treatment from the trial by Jha et al.[2] The authors used a visual analog scale to measure health related quality of life. However, the visual analog scale is known to underestimate quality of life compared to other measurement tools, like for example time trade off and standard gamble. Therefore, we recalibrated these utilities to utility obtained by time-trade off.[16] Literature sources which obtained quality of life estimated with instruments other than VAS scales, were used for all other utility estimates. As the most frequent minor complications are infections due to immunosuppression, we used utility scores for chronic bronchitis as indicators for the quality of life when a patient suffered a minor complication. Similarly, we considered utility scores for a major complication similar to those of neutropenia requiring hospitalization, a transient thrombotic event, hypertension and/or diabetes.

Scenarios on which healthcare related cost estimates were based can be found in the supplementary information online. We recalculated all costs to the consumer price index for 2012.[17] We estimated healthcare related costs for medical procedures from the Dutch hospital reimbursement system (www.nza.nl, accessed

August 2013). In addition, we obtained cost prices of drugs from average consumer reimbursement prices calculated by the Dutch Board for Health Insurance Companies (www.medicijnkosten.nl, accessed August 2013). Both utilities and costs were discounted at a 3.5% rate per year according to National Institute for Health and Care Excellence (NICE) guidelines.

Model validation and sensitivity analyses

Model validation

First, we checked the model's face validity based on its structure. Next, we ran 10 000 simulations for 10 cycles. Subsequently, we obtained ten year probabilities for mortality, end stage renal disease and remission as predicted by the model, and compared these to the actual probabilities observed in the trial by Ponticelli et al.[1] We set the baseline age to 45 years for this analysis to be able to compare the results obtained from the model to the results of the Ponticelli trial.

Sensitivity analyses

We submitted parameters in the model that were not of a stochastic nature but might influence the outcomes to sensitivity analysis in order to explore the uncertainty surrounding these variables. First the age at the time of diagnosis for the reference case was varied with one year intervals between 20 and 80 years in a one-way sensitivity analysis, as we believed that age might be an important modifier of end stage kidney disease risk. Secondly, we varied the annual discount rate for future costs and QALYs gained from 0% to 10% in intervals of 0.1%. We suspected that discounting of long term gains and risks might particularly important when considering the risk and benefits of cyclophosphamide therapy.

We carried out a second order sensitivity analysis by using 1000 patient level Monte Carlo simulations. We modeled parameter uncertainty by taking estimated distributions for all transition probabilities, health utilities and costs. Respective distribution parameters can be found in the supplementary information online. We used second order Monte Carlo simulations to estimate healthcare costs and QALYs gained, and determined the cost effectiveness plane and 95% credibility ellipse. Furthermore, we plotted a cost-effectiveness acceptability curve to assess cost effectiveness of the differing strategies over a range of willingness-to-pay thresholds from €0 to €100 000. Finally, we added perfect test (sensitivity and specificity of 100%) to the second order sensitivity analysis to estimate the acceptable additional cost for cost neutral implementation. We calculated the acceptable cost as:

$$P_{new} = P_{old} + (C_{old} - C_{new}) + WTP(E_{new} - E_{old}),$$

where P_{new} and P_{old} are the prices for perfect test and uβ2m testing, respectively. C_{old} are costs following uβ2m testing, C_{new} costs after the perfect test; *WTP* is willingness to pay threshold, E_{new} and E_{old} stand for effectiveness in terms of QALYs after the perfect test and uβ2m, respectively.

Table 5.2. Model input: rewards for Markov transitions.

State reward	Point estimate	Source
Utilities		
Cancer	0.79	Tengs & Wallace Medical Care 2000[24]: cancer n.o.s.
Complication	0.69	Fryback Medical Decision Making 199325: TTO (Asthma, Chronic bronchitis, chronic sinusitis as proxy for) pneumonia. Tengs and Wallace Medical Care 2000 neutropenia requiring hospitalization, heart disease with transient thrombotic event, hypertension and diabetes.
Death	0	Death is the least desirable state, utility set to 0.
Dialysis	0.50	Liem et al Radiology 2003[23]
Graft loss	0.63	Tengs & Wallace Medical Care 200024: Kidney Transplantation with graft loss 12 months after surgery, returning to dialysis
Kidney transplantation	0.87	Liem et al Radiology 2003[23]
Nephrotic Syndrome	0.74	Jha JASN 2007[9] Mean for the controls
Remission	0.98	Spontenaous remission is the preferred state, utility near perfect
Utility while receiving cyclophosphamide treatment	0.83	Jha JASN 2007[9]: Mean for the intervention group
Healthcare costs (€)		
Annual costs of dialysis	€ 75 064	Annual costs of dialysis include hospital costs and medication
Annual costs of kidney transplantation	€ 35 871	Immunosuppression and outpatient visits
Cancer	€ 11 233	Range obtained from diagnosis and treatment estimates for lung cancer, leukemia/lymphoma, prostate, colon, breast and bladder cancer.

Complications	€ 2 289	Common complications were considered infections and thrombosis. More severe complications were considered as well as these strongly influence health utility and costs. Severe complications were pulmonary embolism, cerebrovascular event and cardiovascular disease related events (angina pectoris and infarction).
Conservative therapy	€ 887	assuming ACEi/ARBs, diuretics and statins
Cyclophosphamide therapy	€ 1 996	
Death	€ 3 487	Two days IC admission
Graft loss	€ 4 619	Assumed to be equal to initial costs of dialysis
Initial costs of dialysis	€ 4 167	Including predialysis visits, medication and placing a shunt
Initial costs of kidney transplantation	€ 61 294	Initial costs include screening of donor and recipient, surgery, and the post transplantation immunosuppression and follow-up

VAS: Visual analog scale. TTO: time-trade-off. VAS is known to underestimate utility when compared to time trade off. Therefore VAS estimates were recalculated into TTO utility estimates: VAS=1-(1-TTO)^α, where α=0.65.[16] *Costs are estimated at the consumer price index for 2012.*[17]

Results

Model validity

Table 5.3 shows the cumulative ten year mortality or end stage renal disease, and remission probabilities predicted by the Markov model. Compared to the study by Ponticelli *et al.*,[1] the model appeared to overestimate mortality and ESRD risk slightly. However, the frequency of events was reasonably similar to that described by Ponticelli and colleagues, and therefore we considered the face validity of the model sufficient to proceed with the cost-effectiveness analyses.

Table 5.3. Model validation: Predicted 10 year outcomes of the base case analysis for the treatment strategies.

Strategy	Mortality and ESRD		Remission	
	Obs	Exp	Obs	Exp
Conservative	36%	31%	28%	36%
Treat all	8%	7%	84%	76%

ESRD: end stage renal disease. Obs: observed probability of the outcome in the Markov model. Exp: expected probability based on the trial by Ponticelli et al.[1]

Base case analysis

Table 5.4 shows the results of the base case analysis. The strategy based on a prognostic test with uβ2m resulted in the lowest mean costs (€19 963) and 13.94 QALYs gained. The KDIGO, universal cyclophosphamide and conservative treatment strategies resulted in higher costs and fewer QALYs gained than the prognostic testing strategy. Therefore, both strategies were considered dominated. In addition, we compared the cost effectiveness of the KDIGO strategy to universal cyclophosphamide treatment. The KDIGO strategy saved €711 compared to treating all patients, but resulted in 0.27 fewer QALYs, giving an ICER of €2 633 per QALY. As the savings were less than €80 000 per QALY, the KDIGO strategy was not cost-effective compared to treating all patients.

Table 4. Cost-effectiveness for four alternative treatment strategies for a 55 year old iMN patient who presented with the nephrotic syndrome.

Strategy	Cost (€)	QALYs gained	ICER
Predict prognosis with uβ2m before treatment allocation	19 963	13.94	
Treat all patients with cyclophosphamide	24 153	13.90	dominated
Treat according to KDIGO guidelines	23 442	13.64	dominated
Conservative treatment	86 186	11.25	dominated

QALY: quality adjusted life year. ICER: incremental cost-effectiveness ratio. Alternatives which were more costly and resulted in fewer QALYs gained were considered 'dominated'. The willingness-to-pay threshold was €80 000 per QALY for the present study.[13] *Therefore, treating all patients with cyclophosphamide was not considered cost-effective.*

Sensitivity analyses

Figure 5.2 shows the cost effectiveness planes for the four strategies obtained from the second order simulations. The scatter plot shows that testing for $u\beta_2m$ prior to deciding upon a treatment remained the most cost effective alternative on average, after taking parameter uncertainty and heterogeneity between patients into account.

Figure 5.3 shows the results for the sensitivity analyses for age at the time of diagnosis, the discount rate for long term costs and QALYs gained. The incremental cost effectiveness ratios are compared to the prognostic testing strategy with $u\beta_2m$. Treating all patients with cyclophophamide was cost-effective when the patient's age at biopsy was higher than 60 years. Both the KDIGO strategy and conservative treatment strategy were dominated throughout the age and discounting ranges. Likewise, treating all patients was cost-effective when the annual discounting rate was over 6.2%.

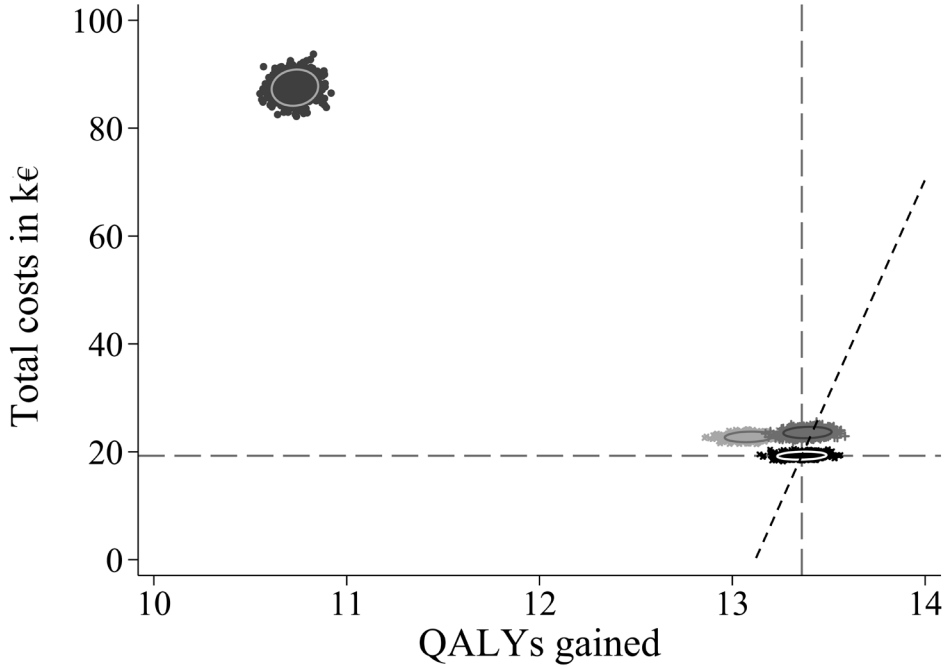

Figure 5.2. Cost effectiveness planes and 95% credibility ellipses for conservative treatment, the KDGIO strategy, universal cyclophosphamide therapy and the prognostic uβ2m test prior to treatment allocation. The long dashed lines are reference lines to determine cost effectiveness compared to the prognostic testing strategy, which was most cost-effective. The short dashed line signifies the willingness-to-pay threshold at €80 000 per QALY gained. At this threshold, both the conservative and KDIGO strategy were dominated by the prognostic testing strategy. The universal cyclophosphamide strategy was cost-effective in 33.9% of the cases. Costs are presented in €1 000 units, QALY: Quality adjusted life year.

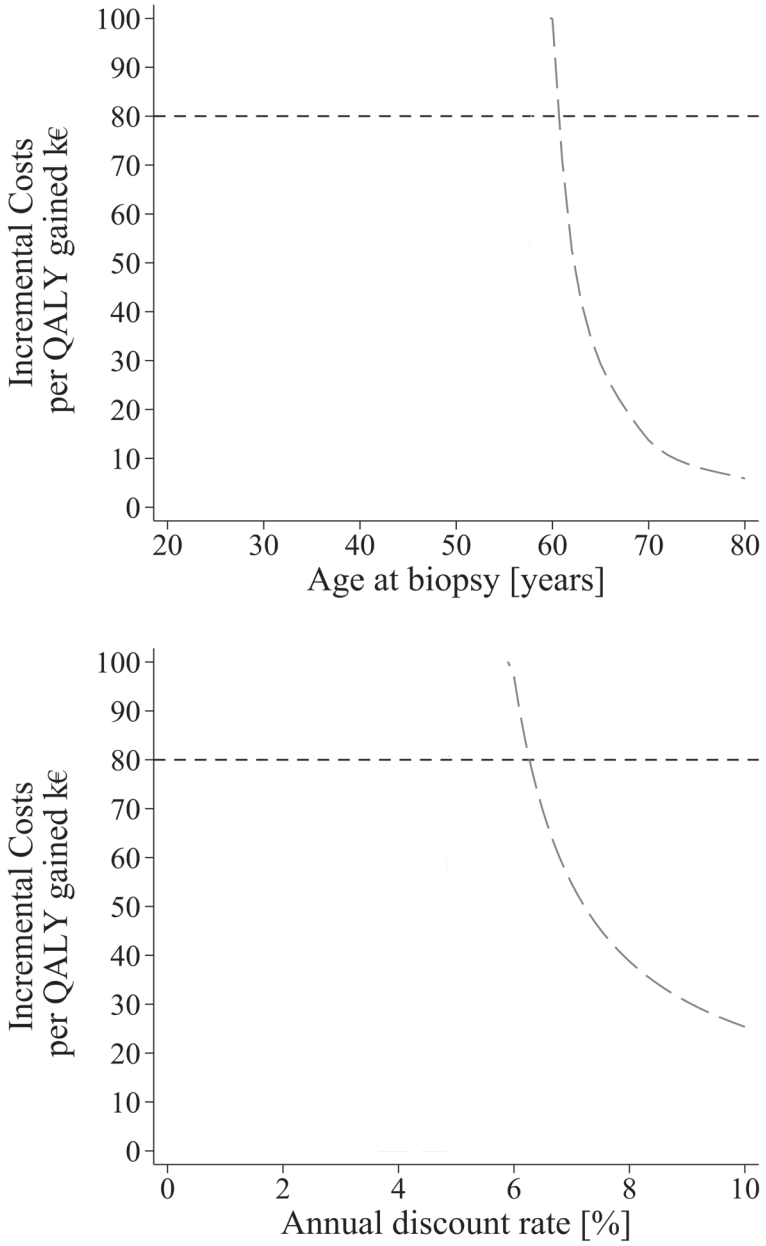

Figure 5.3.One way sensitivity analyses for the incremental cost-effectiveness ratio for universal cyclophosphamide treatment by age (top panel and annual discount rate (bottom panel). The incremental cost effectiveness ratios are compared to the prognostic testing strategy with uβ$_2$m. Both the KDIGO strategy and conservative treatment strategy were dominated throughout the age and discounting ranges, and are therefore not shown. The black short dashed line is the willingness to pay threshold. If the incremental cost-effectiveness ratio are lower than the willingness to pay, the alternative is deemed cost-effective.

The cost effectiveness acceptability curve (figure 5.4) for the restrictive strategy declined with increasing willingness-to-pay, whereas the curve for universal cyclophosphamide increased. However, throughout the willingness-to-pay range, the uβ2m based alternative remained most likely to be acceptable. At a willingness to pay threshold of €80 000 per QALY, the universal cyclophosphamide strategy was the most cost-effective alternative in 35.3% of the simulations.

Finally, a perfect test was added to the second order simulations. Compared to testing for uβ2m excretion, a perfect test would result in €605 saved and 0.07 QALYs gained. Therefore the maximum additional costs for cost neutral implementation would be 605 + 80 000 x 0.07 = €6 205 compared to $u\beta_2m$ testing.

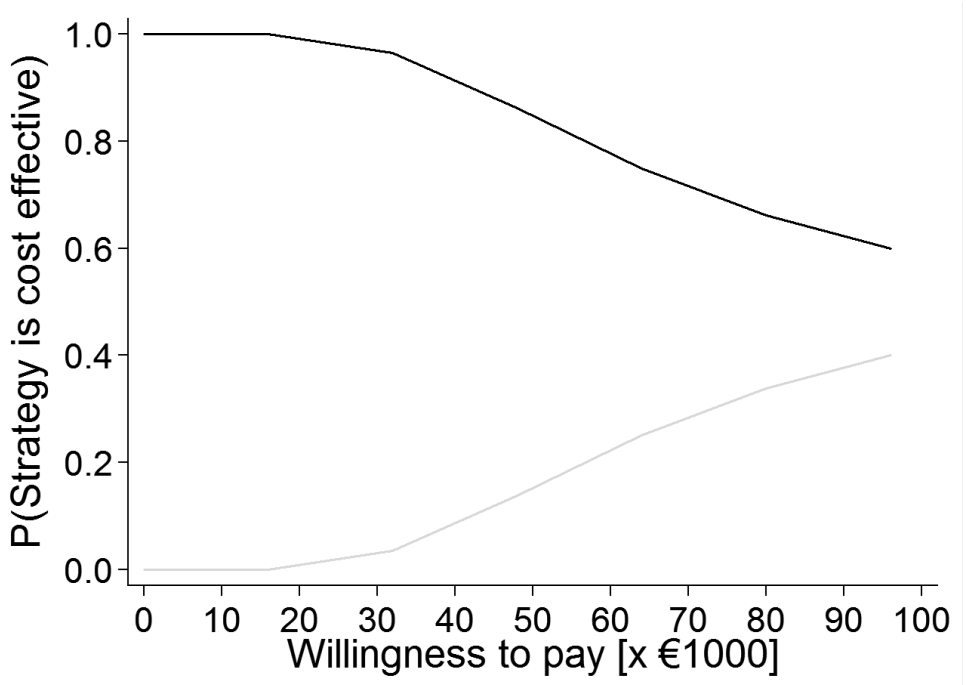

Figure 5.4. Cost-effectiveness acceptability plot. he black line marks the cyclophosphamide therapy; the grey line is the universal cyclophosphamide therapy. The plots shows the probability that a strategy is the most cost effective alternative by varying willingness to pay thresholds. TThe KDIGO strategy and the conservative strategywere never cost-effective. Willingness-to-pay is the maximum extra cost a payer is willing to accept to gain a life year in perfect health.

Discussion

In the present study we describe a cost-effectiveness analysis for four alternative treatment strategies for idiopathic membranous nephropathy in adult patients presenting with nephrotic syndrome and a well preserved kidney function. We compared conservative treatment, treatment according to the KDIGO guidelines, and universal cyclophosphamide therapy to a prolonged watchful waiting strategy based on a prognostic test with urinary β_2-microglobulin excretion.[7] The KDIGO guidelines recommend that treatment should be initiated in patients not showing signs of improving after a watchful waiting period of six to twelve months.[6] Recently, we showed that this watchful waiting period can safely be extended up to approximately three years.[7] Consequently, even fewer patients may be exposed to the possible harmful effects of cyclophosphamide therapy. The present cost-effectiveness study supports this conclusion.

To the best of our knowledge, this is the first study that includes cancer risk after cyclophosphamide treatment as well as cancer risk on dialysis or after kidney transplantation. In addition, we took short and long term complications, ESRD and mortality risk into account. Thus, we give a comprehensive overview of patient outcome beyond the short term consequences of a decision to treat or not to treat. Transition probabilities, healthcare costs and health related utilities were obtained from previous work and available literature, which allowed us to makes realistic estimations and take heterogeneity between patients, and uncertainty surrounding costs, benefits and risks into account. To do so, we performed a series of sensitivity analyses and evaluated the model's robustness.

Remarkably, one way sensitivity analysis for age showed that the differences in costs and effectiveness between the four alternatives decreased with increasing age. In particular, treating all patients with cyclophosphamide became a viable alternative. As the age at which patients are diagnosed with iMN increases, so does the baseline risk of death. In fact the probability of mortality becomes far greater than ESRD and cancer risk in persons aged over 70.[14,18,19] As a result, the relative contribution of living with the nephrotic syndrome to the number of QALYs gained becomes much larger. For example, if a patient spends three years in the nephrotic state, that patients will lose 0.78 QALYs. This is a 7.8% reduction when the life expectancy is 10 years. However, if the life expectancy is three years, almost a third of the QALYs is lost. This would also explain why universal cyclophosphamide therapy is more favorable when discounting rates increase. Here too, the initial gains in QALYs weigh more heavily compared to long term loss of QALYs due to cancer, for example. On the other hand, the risk of short term complications of cytotoxic therapy appears to increase with increasing age.[20] We have not incorporated age dependent complication rates into the Markov model. Therefore, we cannot exclude that the number of QALYs gained in the cyclophosphamide strategy has been overestimated. Additionally, in everyday clinical practice treatment would be started in case of unresponsive nephrotic syndrome or deep hypoalbuminemia. In the present model; however, treatment was either started if spontaneous remission was not achieved (KDIGO strategy) or if serum creatinine increased (patients with low uβ2m who do show progression). Thus the duration of

nephrotic syndrome was likely overestimated in both strategies. In summary, the results for the universal cyclophosphamide strategy may be overly optimistic in this model.

The present study does have some limitations. First, we only modeled the healthcare perspective. Both patient and societal perspectives require more assumptions, adding much uncertainty to the model. As a result, the model would become less reliable, limiting its value. Moreover, our conclusions are unlikely to be influenced by changing the perspective. We expect that adding the patient perspective would result in more costs to be added to cyclophosphamide treatment, as a result of extra travel to the hospital, for example. However, patient costs due to ESRD would be far greater. Dialysis, kidney transplantation and cancer all require frequent hospital visits and thus lead to higher transportation costs and opportunity costs due to work hours lost. Similarly, from a societal perspective, costs due to productivity loss from poor long term outcome are likely to increase the difference in cost-effectiveness between the therapeutic strategies. In the current study, the healthcare perspective offered a conservative estimate of difference in cost effectiveness between strategies. Second, the costs have been estimated in the Dutch setting. Treatments may be similar in other Western settings, costs may not. However, renal replacement therapy and cancer treatment are very costly regardless of the setting. Moreover, in countries where access to both renal replacement therapy and oncologic care is limited, the restrictive uβ_2m strategy is even more likely to result in a larger amount of QALYs gained compared to the other alternatives. Therefore, the results from the present study are likely to be generalizable to other countries. Third, we only modeled cyclophosphamide based therapeutic strategies, as alkylating agents have been shown to be efficacious in randomized controlled trials, and are thus recommended by KDIGO guidelines. Inferences from cyclophosphamide strategies do not necessarily generalize to other therapeutic regimens. Moreover, some assumptions were made to simplify the Markov model. First and foremost, the consequences of complications and cancer were modeled as being immediate. As a result the number QALYs gained in persons who died from cancer or complications were likely underestimated. Conversely, QALYs accrued by patients who survived cancer or complications may have been overestimated. Finally, estimates for transition probabilities, utilities and costs were not based on an exhaustive literature review. However, we believe that the most important literature in the field of membranous nephropathy has been covered. Nevertheless, the model uncertainty may in fact be somewhat larger than estimated in the sensitivity analyses.

In addition to comparing currently available treatment strategies, we calculated the price at which a perfect prognostic test can be implemented cost neutrally. A test close to 100% sensitivity and specificity could be implemented at a price of €6 000 per patient, or $5 200 in the USA, and £2 100 in the UK. Therefore commercial assay development may be worthwhile. Finally, we reported the analyses in such a way that they can be used as a framework by others to determine the cost effectiveness of novel prognostic markers and new therapeutic options in the treatment of iMN.

Conclusion

Restrictive cyclophosphamide therapy based on a prognostic test with urinary β_2-microglobulin resulted in lower costs and more quality adjusted life years gained than the therapy currently recommended in the KDIGO guidelines. The model presented here may be used as a framework to evaluate future prognostic markers and treatments.

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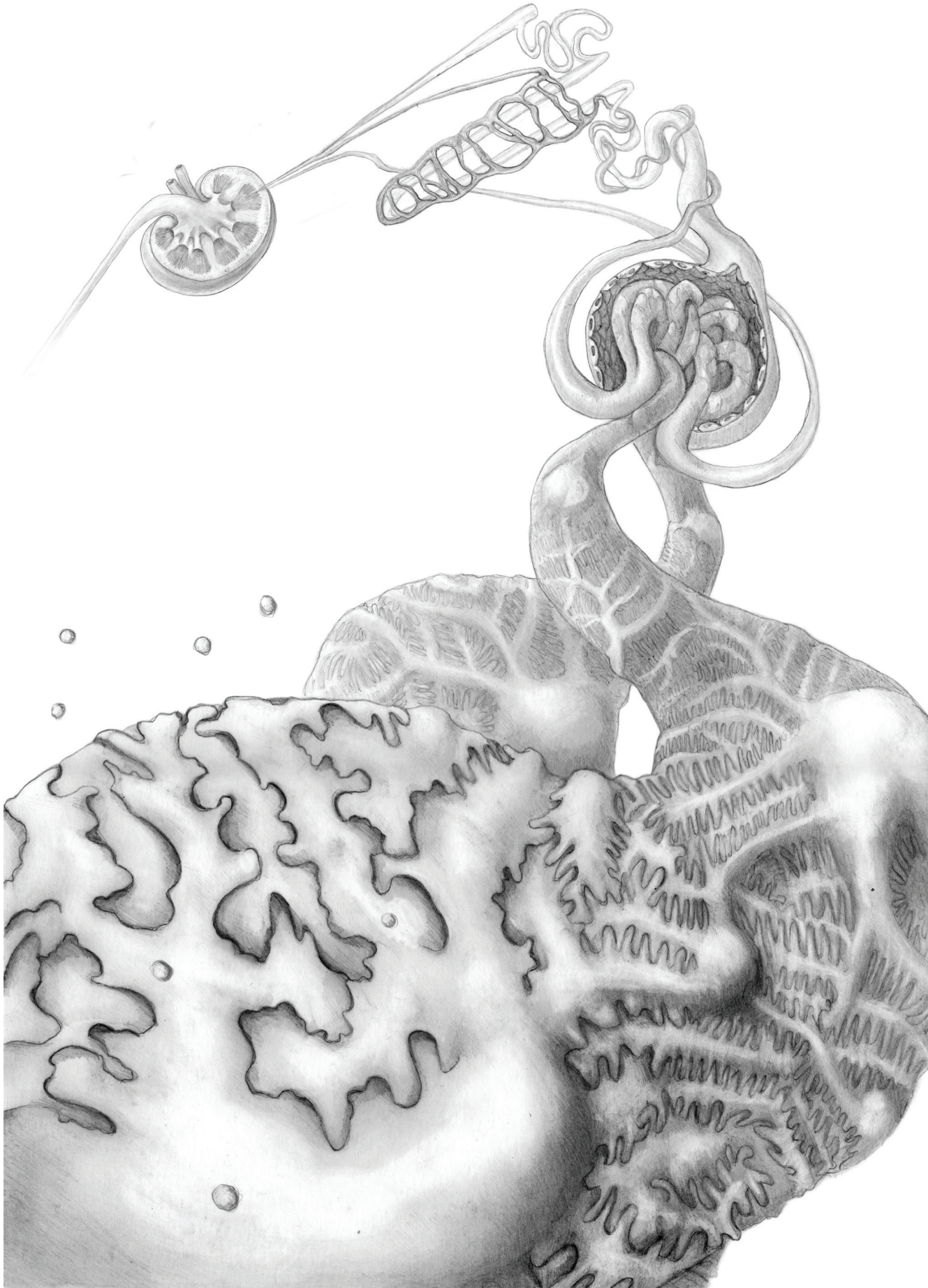

Chapter 6: Summary and perspectives

Summary

As described in *chapter 1*, idiopathic membranous nephropathy (iMN) is the most common cause of nephrotic syndrome in adults. The prognosis of iMN is variable. Without immunosuppressive therapy up to half of all nephrotic patients will show progressive kidney function loss, and ultimately require renal replacement therapy. Both dialysis and kidney transplantation have a great impact on a patient's health and quality of life, and are very costly. Immunosuppressive therapy with alkylating agents has been proven effective. Unfortunately these drugs are associated with severe side effects. Up to half of all patients with nephrotic syndrome may develop spontaneous remission of proteinuria. Therefore international guidelines recommend that immunosuppressive treatment should be restricted to patients at the highest risk of progressive kidney failure. According to these guidelines, high risk patients are those who have nephrotic syndrome for six to twelve months, or a deteriorating kidney function. However, these recommendations are based on very little evidence. The following questions arise and were addressed in this thesis:

1. How well can we predict prognosis in idiopathic membranous nephropathy?
2. Can we improve prediction of prognosis?
3. Is restrictive therapy with cyclophosphamide effective?
4. What is the malignancy risk of cyclophosphamide therapy?
5. Do the benefits of restrictive cyclophosphamide therapy outweigh the risks and costs?

In order to answer these questions, epidemiological studies and a decision analysis have been performed based on data collected in our prospective registry of patients with glomerular diseases. An introduction of the methodology is given in *chapter 2*.

Chapter 3 includes two studies on the prediction of prognosis in iMN. In the first study (*chapter 3.1*) we validated the prognostic accuracy of urinary excretion of low molecular weight markers β_2-microglobulin and α_1-microglobulin in a cohort of 129 patients who presented to our clinic with a well preserved kidney function and nephrotic syndrome. We showed that the rate of urinary excretion of these low molecular weight proteins can discriminate with 80% accuracy between patients who show progressive kidney function loss and those who do not. Moreover, we concluded that a cumbersome timed urine analysis may not be needed. Our data indicate that spot urine samples, and subsequent calculation of the ratio of urinary low molecular weight proteins and creatinine can suffice. More importantly, in an exploratory analysis of a subgroup of 44 patients, we showed that a repeated measurement of β_2-microglobulin within six to twelve months may improve its accuracy. Progression risk was 100% if β_2-microglobulin excretion was over 1.0 µg/min at both measurements. Likewise, progression risk was about 80% when β_2-microglobulin excretion was elevated once. If both values were below 1.0 µg/min progression did not occur at all. The use of repeated measurements still requires validation. In the second study (*chapter 3.2*) we compared the predictive power of urinary excretion of low molecular weight proteins to an algorithm that is often used as an alternative, the Toronto Risk Score. This algorithm predicts prognosis

based on creatinine clearance and the level of proteinuria over a follow-up period of six months during which proteinuria is at its highest level. We did not find a substantial difference in accuracy. Interestingly, we found that not the level of proteinuria, but rather creatinine clearance and its change over the course of six months were the biggest contributors to the prognostic power of the Toronto Risk Score. As creatinine clearance does not accurately reflect glomerular filtration rate in patients suffering from the nephrotic syndrome, we adapted the Toronto Risk Score to include the estimated glomerular filtration rate according to the six variable version of the Modification of Diet in Renal Disease equation. Finally, we showed that, instead of waiting for the period of maximum proteinuria, the Toronto Risk Score can be calculated during the first six months of follow-up and still provide a reasonably accurate prediction of prognosis.

In *Chapter 4*, we describe two studies on the long term outcome of iMN patients who were treated according to a restrictive regimen practiced at the Radboud university medical center since the late 1990s. Based on close monitoring of serum creatinine levels of patients with iMN who presented with nephrotic syndrome, patients who showed initial progression were identified and treated with immunosuppressive drugs, whereas low risk patients received conservative treatment only. We studied the long term outcome of 254 patients who were referred to our centre and treated according to this restrictive regimen. The results are described in *chapter 4.1*. Only 124 of the 254 (49%) patients were treated with immunosuppressive drugs. Overall, 3% of all patients required renal replacement therapy and 10% died during follow-up. By comparison, 8% of the treated patients in the trial by Ponticelli and coworkers suffered one of these outcomes, and 40% of the untreated patients in that trial needed dialysis or died. In other words, our restrictive treatment strategy gave results similar to a regimen that administered immunosuppressive drugs to all patients, while sparing half of the patients from toxic treatment. Importantly, our results suggest that the watchful waiting period prior to initiating immunosuppressive treatment can be safely extended to up three years. Almost 70% of the supportively treated patients developed a spontaneous remission of proteinuria during that time. In addition, one thirds of all patients who were treated with immunosuppression had a serious adverse event, which resulted in a hospitalization in 40% of these patients. The most notable complications were low white blood cell count, and infections as a consequence. Notably, the incidence of malignancies appeared to be increased in patients who were treated with cyclophoshamide. In a follow-up study, detailed in *chapter 4.2*, we quantified the cancer risk following cyclophosphamide treatment in patients with iMN. In total 272 patients were included, 127 of whom were treated with cyclophosphamide. The crude cancer incidence was 21.2 per 1000 person years in treated compared to 4.6 per 1000 person years in untreated patients. This resulted in an incidence ratio of 4.6 with a 95% confidence interval of 1.5 to 18.8. When we took confounding factors into account, the incidence ratio was attenuated to 3.2 (95% confidence interval: 1.0 to 9.5). For an average patient, presenting at the age of 55, this would mean that the annual cancer risk increases from 0.3% to 1.0%.

Finally, we used the results of these studies and data gathered from the literature to create a decision model in which we weighed the risks and benefits of the restrictive cyclophosphamide therapy, detailed in *chapter 5*. We compared four

alternative treatment strategies, being:
1. Treating all with cyclophosphamide (according to the Ponticelli regimen);
2. Treating patients as recommended by international guidelines;
3. Treating only high risk patients identified by a prognostic test with urinary β2-microglobilin;
4. Treating all patients conservatively with ACEi/ARBs.

The decision analysis showed that the restrictive cyclophosphamide regimen based on the prognostic test resulted in the highest number of quality adjusted life years gained at the lowest costs.

In conclusion, by using currently available prognostic tools such a urinary β_2- or α_1-microglobulin or the Toronto Risk Score, we can predict the prognosis in patients with iMN who present with nephrotic syndrome with approximately 80% accuracy. Repeated measurements of urinary low-molecular weight proteins within the first year of follow-up may substantially improve prognostic accuracy. The restrictive treatment strategy is effective and spares half of all iMN patients from possible treatment related side effects. In total, one in three patients who receive cyclophosphamide suffers an adverse event. The most notable complications are leucopenia, infections and cancer. The cancer risk is three times higher compared to untreated patients. Finally, decision analysis showed that a restrictive regimen based on a prognostic test with urinary β_2-microglobulin and an extended watchful waiting period was the most cost-effective alternative compared to treating all patients with cyclophosphamide, treatment according to current international guidelines and treating all patients conservatively.

Future perspectives in idiopathic membranous nephropathy

A major change in the field of iMN occurred while performing the studies described in this thesis. As mentioned in *chapter 1*, the discovery of the phospholipase A2 receptor (PLA2R) as the target antigen in iMN has opened to door to new opportunities and insights regarding diagnosis, prognosis and treatment.[1,2] It is now well established that 70% of all patients with iMN have circulating auto-antibodies for PLA2R.[1] In fact, the concept of 'idiopathic membranous nephropathy' as a disease may be outdated, instead it should be considered a kidney specific auto immune disease. Measurements of antibodies against PLA2R, either circulating or in a kidney biopsy, will aid the diagnosis of membranous nephropathy, and can also be used to exclude secondary causes of MN. Moreover, antibody assays may be valuable in predicting prognosis and guiding therapy. Hofstra et al. showed that the titer of circulating anti-PLA2R antibodies correlated well with the presence of proteinuria and clinical status.[3] In a later study, Beck et al. showed that treatment with rituximab resulted in decreasing titers of anti-PLA2R antibodies which preceded a decrease in proteinuria.[4] More recently, a collaborative European study showed that patients with high levels of auto-antibodies were less likely to develop a spontaneous remission of proteinuria.[5] Additionally, a pilot study showed that persisting presence of anti-PLA2R antibodies in serum of patients during treatment predicted worse outcome.[6] Moreover, antibodies disappeared at a lower rate when patients were treated with mycophenolate mofetil compared to cyclophosphamide. In turn,

outcome in the mycophenolate mofetil treated patients was worse compared to the cyclophosphamide patients. In conclusion, measuring anti-PLA2R antibodies may be helpful in predicting prognosis, deciding on treatment start and guiding the duration of immunosuppressive therapy.

Even though evidence has only recently been provided, idiopathic membranous nephropathy has long been hypothesized to be an auto-immune disease. Therefore, immunosuppressive treatment has been considered since the 1980s. Cytotoxic agents such as cyclophosphamide have unequivocally been shown to be effective in randomized controlled trials,[7-9] and are therefore recommended as first line immunosuppressive treatment in iMN.[10] However, the serious side effects of cyclophosphamide therapy necessitate the development of new, effective and less toxic therapies. Therefore, the group of Remuzzi started experimental treatments with rituximab at the turn of the century.[11] Rituximab is a monoclonal anti-body against the CD20 receptor found on B lymphocytes. It has already been used in 100 consecutive patients in the Italian cohort, and with apparent success.[12] However, it was an uncontrolled study, and therefore causal inferences cannot be reliably made. A formal comparison between rituximab and cyclosporin A is currently underway in the USA (clinicaltrials.gov, NCT01180036). In addition to that trial, a direct comparison between cyclophosphamide, the current standard therapy, and rituximab is needed as well.

In conclusion, the treatment of idiopathic membranous nephropathy is set to change in the near future. Anti-PLA2R antibody titers may be used to identify which patients to treat and for how long. Secondly, new treatments such as rituximab may offer a viable alternative to cyclophosphamide. The first data has only recently started to emerge. More extensive and robust studies are going to be needed to provide evidence to support these novel treatment regimens.

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Chapter 7: Samenvatting

Summary in Dutch

Idiopathische membraneuze nefropathie is een ernstige nierziekte. Het is de meest voorkomende oorzaak van nefrotisch syndroom bij volwassenen. Patiënten die lijden aan nefrotisch syndroom verliezen grote hoeveelheden eiwit via de urine. Dit komt doordat antilichamen neerslaan in de de glomeruli, de filtereenheden van de nier. De glomeruli raken dan beschadigd. Eiwitten die normaal in de bloedbaan blijven lekken door de beschadigde filter naar de voorurine. Het natuurlijk beloop van membraneuze nefropathie wisselt sterk. In de helft van de gevallen gaan de symptomen vanzelf over. We spreken dan van een spontane remissie. In de andere helft van de gevallen treedt verdere schade op. Uiteindelijk verliezen deze patiënten hun nierfunctie helemaal. Dan hebben zij dialyse behandeling of een niertransplantatie nodig.

Behandeling met afweeronderdrukkende medicijnen is bewezen effectief om nierschade te voorkomen en nefrotisch syndroom in remissie te laten gaan. Een van de belangrijkste afweeronderdrukkende middelen is cyclophosphamide. Helaas heeft cyclophosphamide soms ernstige bijwerkingen. Behandeling kan bijvoorbeeld leiden tot een verhoogd risico op kanker. Daarom gaan we er vanuit dat het beperken van de behandeling tot patiënten bij wie we verwachten dat de nierfunctie achteruitgaat, de beste strategie is. Om na te gaan of dit echt zo is, moeten we de volgende vragen beantwoorden:

1. Hoe goed kunnen we voorspellen bij welke patiënten de nierfunctie achteruit zal gaan?
2. Kunnen we deze voorspelling verbeteren?
3. Is het beperken van afweeronderdrukkende behandeling tot patiënten met een hoog risico op nierfunctieverlies effectief?
4. Hoe groot is het risico op kanker na behandeling met cyclophosphamide?
5. Wegen de voordelen van het beperken van cyclophosphamide behandeling op tegen de mogelijke nadelen en kosten?

Om deze vragen te beantwoorden hebben we een aantal epidemiologische studies en een besliskundige analyse uitgevoerd. In *hoofdstuk 2* beschrijven we de basis principes van deze vormen van onderzoek.

In *hoofdstuk 3* beschrijven we twee studies waarin we de prognose van patiënten voorspellen. We hebben de urineuitscheiding van kleine eiwitten gemeten bij alle patiënten die verwezen zijn naar het Radboud universitair medisch centrum. We meten de uitscheiding van deze kleine eiwitten, α_1-microglobuline en β_2-microglobuline, met een getimede urine meting. We laten in *hoofdstuk 3.1* zien dat we kort na diagnose met 80% zekerheid kunnen voorspellen of een patiënt nierfunctie verlies zal ontwikkelen of niet. Dat is een duidelijke verbetering ten opzichte van de *fifty-fifty* kans die een patiënt voor de meting heeft. Daarnaast laten we zien dat de ingewikkelde, getimede urine meting niet nodig is. In plaats daarvan bepalen we α_1- of β_2-microglobuline concentratie en delen deze door de kreatinine concentratie in het zelfde urine monster. Kreatinine is een merkstof, gemaakt in de spieren, die met constante snelheid door de nier wordt uitgescheiden. Daarom kunnen we de concentratie van kreatinine gebruiken om te corrigeren voor verdunning van de urine. Tenslotte laten we zien dat we de nauwkeurigheid van onze voorspelling verder kunnen verbeteren door de meting van β_2-microglobuline na zes tot twaalf maanden te herhalen. Patiënten bij wie de uitscheiding bij beide metingen verhoogd is, verliezen nierfunctie. Tegengesteld,

patiënten bij wie de uitscheiding van β2-microglobuline bij beide metingen niet verhoogd is, hebben geen nierfunctieverlies.

In *hoofdstuk 3.2* vergelijken we de voorspelling aan de hand van kleine urine eiwitten met een veelgebruikt algoritme, de Toronto Risico Score. Deze risico score gebruikt de hoogte van eiwitverlies en kreatinineklaring, een maat voor nierfunctie, om een schatting te maken van de prognose. In tegenstelling tot het meten van kleine eiwitten, is een lange vervolg periode nodig om de Toronto Risico Score te kunnen berekenen. Toch geven dit algoritme en de uitscheiding van α_1- en β_2-microglobuline een even goede voorspelling van de prognose. We laten zien dat de kreatinineklaring en niet het eiwitverlies het meeste bijdraagt aan de voorspelling door de Toronto Risico Score. Omdat kreatinineklaring geen goede maat is voor nierfunctie bij mensen met nefrotisch syndroom, hebben we vervolgens een betere schatting van nierfunctie gebruikt om de Toronto Risico Score te herzien. Tevens hebben we alleen gegevens verzameld tijdens de eerste zes maanden na diagnose gebruikt om nierfunctie verlies te voorspellen. We laten zien dat daarmee een vroegere maar even goede voorspelling van de prognose mogelijk is.

Naast de studies naar de prognose van iMN hebben we bekeken of het beperken van behandeling met afweeronderdrukkende medicijnen tot patiënten die het meeste risico lopen op nierfalen even goede resultaten geeft als alle patiënten met deze middelen behandelen. In *hoofdstuk 4.1* beschrijven we de lange termijn uitkomsten van restrictieve behandeling. Volgens dit regime krijgen alleen patiënten met nierfunctieachteruitgang of ernstig nefrotisch syndroom afweeronderdrukkende therapie met cyclophosphamide. Sinds 1995 zijn 254 patiënten die zijn verwezen naar het Radboudumc behandeld volgens het restrictieve regime. Slechts de helft van deze patiënten heeft uiteindelijk cyclophosphamide gekregen. De tien jaars overleving van alle patiënten samen was 90%. Drie procent van de patiënten had nierfunctievervangende therapie nodig. Ter vergelijking, in studies waarin alle patiënten werden behandeld overleefde 92% van de patiënten zonder noodzaak tot nierfunctievervanging. Met andere woorden, het restrictieve regime geeft vrijwel dezelfde overlevingswinst, maar spaart bij de helft van de patiënten behandeling met afweeronderdrukkende medicijnen uit. Ook blijkt dat we behandeling veilig kunnen uitstellen tot drie jaar na diagnose.

Behandeling met afweeronderdrukkende medicijnen geeft bijwerkingen. Cyclophosphamide therapie geeft risico op een tekort aan witte bloedcellen en daarmee een grotere kans op infecties. Dit leidde tot ziekenhuisopname in 13% van de behandelde patiënten. Een andere, belangrijke bijwerking is een verhoogd risico op kanker. In *hoofdstuk 4.2* beschrijven we een studie waarin we maat en getal aan dat risico geven. In deze studie hebben we 272 patiënten gevolgd waarvan er 127 hebben cyclophosphamide behandeling kregen. We laten zien dat de kans op kanker verdrievoudigd na behandeling met cyclophosphamide. Dit betekent dat voor een patiënt van 55 jaar het risico op kanker toeneemt van 0.3% tot 1.0% per jaar.

Met de gegevens uit voorgaande studies en resultaten van andere wetenschappers is het mogelijk om een besliskundige analyse uit te voeren. We hebben in *hoofdstuk 5* een simulatiestudie uitgevoerd waarin we vier mogelijke

behandelstrategieën met elkaar vergelijken:
1. Iedereen behandelen met cyclophosphamide.
2. Behandeling volgens internationale richtlijnen.
3. Behandeling volgens het restrictieve regime beschreven in *hoofdstuk 4.1*.
4. Conservatieve behandeling met bloeddruk verlagende middelen.

Internationale richtlijnen schrijven voor dat alleen patiënten die zes tot twaalf maanden na diagnose nog steeds nefrotisch syndroom hebben cyclophosphamide therapie hoeven te krijgen. Patiënten bij wie de ziekte gedurende deze periode vanzelf in remissie gaat, behoeven alleen ondersteunende behandeling. Het is dus ook een restrictieve behandeling, maar er wordt minder lang gewacht voordat afweeronderdrukkende medicijnen worden voorgeschreven dan in het regime van het Radboudumc.

Uit onze analyse blijkt dat behandelen volgens het restrictieve regime van het Radboudumc de meeste gewonnen levensjaren in volledige gezondheid oplevert tegen de laagste kosten.

Tenslotte bespreken we in *hoofdstuk 6* ook de toekomstperspectieven voor het onderzoek naar en de behandeling van idiopathische membraneuze nefropathie. De ziekte was decennialang 'idiopathisch,' dat wil zeggen dat de exacte oorzaak onbekend is. Echter, recent is ontdekt dat 70% van alle patiënten met idiopathische membraneuze nefropathie antistoffen hebben tegen de fosfolipase A2 (PLA2) receptor. Deze receptor is bij patiënten te vinden op de podocyt, een van de belangrijke cellen in de glomeruli. Dit betekent dat idiopathische membraneuze nefropathie in feite een auto-immuun ziekte is. Het afweersysteem maakt antistoffen aan tegen de lichaamseigen PLA2 receptor. Bij patiënten met actieve ziekte circuleren antistoffen tegen de PLA2 receptor in de bloedbaan. Verschillende onderzoekers laten zien dat afname van de antistofconcentratie door afweeronderdrukkende behandeling vooraf gaat aan remissie van nefrotisch syndroom. Het bepalen van PLA2 antilichaamconcentraties zou in de nabije toekomst kunnen helpen om de behandeling van membraneuze nefropathie verder te personaliseren. Een tweede interessante ontwikkeling is het gebruik van rituximab als behandeling in idiopathische membraneuze nefropathie. Rituximab is een antistof tegen B-cellen, de voorlopers van plasmacellen. Plasmacellen maken antistoffen. Dus door B-cellen uit te schakelen zou ook de productie van antistoffen tegen de PLA2 receptor verdwijnen en de ziekte in remissie gaan. De eerste resultaten zijn veel belovend, maar er zijn nog geen gecontroleerde studies uitgevoerd. Een directe vergelijking tussen rituximab en restrictieve cyclophosphamide behandeling moet nog volgen. Tot die tijd blijft cyclophosphamide behandeling de standaard behandeling bij hoog risico patienten.

In conclusie: we kunnen beschikbare merkstoffen, zoals β_2-microglobuline, gebruiken om te voorspellen welke patiënten hoog risico lopen op nierfalen. We hoeven alleen deze hoog risico patiënten te behandelen met afweeronderdrukkende medicijnen. Zo doende beperken we het risico op bijwerkingen en halen we goede lange termijn resultaten. Deze aanpak levert de meeste gewonnen levensjaren in volledige gezondheid op, tegen de laagste kosten.

Chapter 8: Dankwoord, Publication List, PhD Training Overview and Curriculum Vitae

Dankwoord

Als eerste wil ik mijn dank betuigen aan patiënten, medische en administratieve staf van de afdelingen nierziekten of interne geneeskunde van de ziekenhuizen die hebben meegewerkt aan de registratie van patiënten met glomerulaire ziekten. Met uw hulp hebben we de behandeling van patiënten met membraneuze nefropathie de afgelopen jaren weten te verbeteren. Verder wil ik u bedanken voor de gastvrijheid waarmee mijn collaga's en ik zijn ontvangen wanneer we op bezoek kwamen om data te verzamelen uit medische statussen.

Ten tweede mijn promotor, professor doctor Jack Wetzels. Toen ik mijn epidemiologie stage bij de afdeling nierziekten liep, was ik diep onder de indruk van hoe veel jij uit de meest eenvoudige beschrijving van data wist af te lezen. Ik realiseerde me hoe veel ik zou kunnen leren van jou. Dat geldt na ruim 5 jaar onderzoek onder jouw supervisie nog steeds. Ik wil je bedanken voor je geduld -ik kan af en toe koppig en slordig zijn. Jouw toewijding en onaflatende streven naar het best mogelijke onderzoek zijn inspirerend. Je hebt mij altijd weten te motiveren om niets dan het beste te accepteren. Ik geniet nog steeds van de overleggen waarin we mijmeren over de staat van nieronderzoek en filosoferen over welke kant dat onderzoek op zou moeten gaan. Ik hoop dat we nog lang kunnen samenwerken en dat ik nog veel meer van je mag leren.

Ik wil doctor Julia Hofstra bedanken. De manier waarop jij klinisch werk, toponderzoek en moederschap weet te combineren is ongelooflijk. Je bent een ontzettend intelligente en getalenteerde onderzoeker, veel meer dan je zelf toegeeft. Toen ik een paar jaar geleden -vlak na jouw promotie, vroeg of je interesse had om mijn copromotor te worden, zei je van niet. Je zou naar eigen zeggen niet genoeg bijdragen. Ik ben blij dat je van gedachten bent veranderd. Je bent bij ieder onderzoek in mijn proefschrift betrokken en hebt ieder manuscript in dit boekje vele malen gelezen en verbeterd. Als dat niet genoeg is, weet ik niet wanneer het ooit genoeg zou zijn. Ik kijk uit naar je toekomstige werk.

Ik wil in het bijzonder professor doctor Martin den Heijer bedanken. Je bent zonder twijfel de meest vriendelijke persoon die ik ooit heb ontmoet. Mijn promotietraject is er heel anders uit komen te zien dan gepland. Je hebt het Radboud ziekenhuis verlaten voor een hoogleraarschap aan het VU medisch centrum aan het begin van mijn promotieonderzoek. Toch heb je in de korte tijd ik met je heb mogen samenwerken een flinke impact gehad. Dankzijn jou ruilde ik SPSS in voor Stata. Je hebt me aangemoedigd om veel nieuwe technieken en methodes te proberen. Dat resulteerde in aanklooien en heel veel fouten maken voordat iets lukte. Uiteindelijk heb ik van iedere fout geleerd en alles wat de eerste keer mislukte, heb ik in latere studies succesvol kunnen toepassen. Ten slotte heb je mij kennis laten maken met het Nijmegen Centre for Evidence Based Practice, nu het Radboud Institute for Health Sciences, en een Trainings- en Supervisieplan. Geen plan overleeft het eerste contact met de vijand, maar het heeft wel geholpen in de rest van mijn promotie traject.

Hilde, Rutger, Ilse, Annemiek en Simone en alle anderen die de urinemetingen uit hebben gevoerd en nog steeds doen. Zonder jullie harde werk zou dit proefschrift nooit tot stand zijn gekomen, bedankt.

Peter van Dijk, je heb de bulk van data verzameld voor de studies in hoodstuk 4. Bedankt voor je bijdrage. Ik weet zeker dat je een goede dokter en wetenschapper zult worden. Veel succes met je eigen promotieonderzoek.

Louis Reichert, Amanda Branten en Peggy Du Buf- Vereijken. “Als ik verder hebben kunnen zien, dan was dat omdat ik op schouders van reuzen stond.” Jullie zijn mijn reuzen. Gerard Du Buf, ik ken je niet persoonlijk, maar heb wel de vruchten van jouw werk geplukt. Bedankt dat je de *Proteinurie database* hebt gebouwd. We hebben er bijna twee decennia van genoten, maar we gaan hem nu toch echt vervangen.

Ik wil mijn zus, Hanny van den Brand bedanken voor de prachtige illustraties die zij heeft gemaakt. Je hebt van mijn vage ideeën fantastische schetsen gemaakt. Hopelijk is deze eerste opdracht een mooie opmaat naar je cariere als wetenschappelijk illustrator

Ik wil alle leden van de NCEBP PhD council met wie ik het plezier heb gehad samen te mogen samenwerken bedanken. Jullie waren een leuke afleiding in het alledaagse leven van een promovendus. Ik wil ook de leden van het management team van het NCEBP bedanken: Gerdi Egberink, Marieke de Visser, Karin Berens, Paul Smits en Bart Kiemeney. Jullie hebben me een uniek kijkje in de keuken van een groot onderzoeksinstituut gegeven. Ongetwijfeld zal die ervaring helpen bij mijn carrière in de wetenschap.

Natalie, Elke, Christine, Pierre, Olivier, Christophe, Ross, Hans, Andy and Dick. Without exception, you guys make the ASN Kidney Weeks and ERA-EDTA workshops happy reunions. I hope to being seeing a lot more of you in the coming years. Good luck on your studies.

Ik wil alle mensen van wie ik heb mogen leren en met wie ik heb samengewerkt in de afgelopen paar jaar bedanken. Ik had jullie bij naam moeten noemen, mijn excuses dat ik dat niet heb gedaan.

Pa en ma, bedankt voor de goede opvoeding die jullie me hebben gegeven. Ik heb het niet altijd makkelijk gemaakt, maar gelukkig hebben jullie me een flinke portie gezond verstand bijgebracht. Bedankt dat jullie altijd voor me klaar staan. Ik ben trots op jullie en hou van jullie.

Ten slotte, bedankt Kim. Ik zou met gemak een boek ter grootte van dit manuscript kunnen schrijven over wat je voor me betekent. Dat is niet nodig. Alles wat ik te zeggen heb, kan toch niet met woorden worden gevangen.

Other publications by the author

1 **Nurse Practitioner Care Improves Renal Outcome in Patients with CKD**

Mieke J. Peeters, Arjan D. van Zuilen, *Jan A.J.G. van den Brand*, Michiel L. Bots, Marjolijn van Buren, Marc A.G.J. ten Dam, Karin A.H. Kaasjager, Gerry Ligtenberg, Yvo W.J. Sijpkens, Henk E. Sluiter, Peter J.G. van de Ven, Gerald Vervoort, Louis-Jean Vleming, Peter J. Blankestijn, and Jack F.M. Wetzels

Journal of the American Society of Nephrology Volume: 25 Issue: 2 Pages: 390-398 Published: 2014

2 **A retrospective study of focal segmental glomerulosclerosis: clinical criteria can identify patients at high risk for recurrent disease after first renal transplantation**

Maas, R. J. H.; Deegens, J. K. J.; *van den Brand, J.A.J.G.*; Cornelissen, E.A.M; Wetzels, J.F.M.

Bmc Nephrology Volume: 14 Published: 2013

3 **Validation of the kidney failure risk equation in European CKD patients**

Peeters, Mieke J.; van Zuilen, Arjan D.; *van den Brand, Jan A. J. G.*; Bots, M.L.; Blankestijn, P.J.; Wetzels, J.F.M. on behalf of the MASTERPLAN study group.

Nephrology Dialysis Transplantation Volume: 28 Issue: 7 Pages: 1773-1779 Published: 2013

4 **Differences between hospitals in attainment of parathyroid hormone treatment targets in chronic kidney disease do not reflect differences in quality of care**

Peeters, M. J.; van Zuilen, A. D.; *van den Brand, J.A.J.G.*; Blankestijn, P.J.; Wetzels, J.F.M.

Bmc Nephrology Volume: 13 Published: 2012

6 **Epidemiology of Contrast Material-induced Nephropathy in the Era of Hydration**

Balemans, Corinne E. A.; Reichert, Louis J. M.; van Schelven, Bert I. H.; *van den Brand, J.A.J.G.*; Wetzels, J.F.M.

Radiology Volume: 263 Issue: 3 Pages: 706-713 Published: 2012

7 **Intra-individual variability of serum hepcidin-25 in haemodialysis patients using mass spectrometry and ELISA**

Peters, H. P. E.; Rumjon, A.; Bansal, S. S.; Laarakkers, C.M.; *van den Brand, J.A.J.G.*; Sarafidis, P.; Musto, R.; Malyszko, J.; Swinkels, D.W.; Wetzels, J.F.M ; Macdougall, I.C.

Nephrology Dialysis Transplantation Volume: 27 Issue: 10 Pages: 3923-3929 Published: 2012

8 **Segmental and Global Subclasses of Class IV Lupus Nephritis Have Similar Renal Outcomes**

Haring, C. M.; Rietveld, A.; van den Brand, J.A.J.G.; Berden, J.H..

Journal of the American Society of Nephrology Volume: 23 Issue: 1 Pages: 149-154 Published: 2012

9 **High urinary excretion of kidney injury molecule-1 is an independent predictor of end-stage renal disease in patients with IgA nephropathy**

Peters, Hilde P. E.; Waanders, Femke; Meijer, Esther; *van den Brand, J.A.J.G.*; Steenbergen E.J.; van Goor, H.; Wetzels, J.F.M.

Nephrology Dialysis Transplantation Volume: 26 Issue: 11 Pages: 3581-3588 Published: 2011

10 **Introduction of the CKD-EPI equation to estimate glomerular filtration rate in a Caucasian population**

van den Brand, J.A.J.G.; van Boekel, G. A. J.; Willems, H. L.; Kiemeney, L.A.L.M.; Den Heijer, M; Wetzels, J.F.M.

Nephrology Dialysis Transplantation Volume: 26 Issue: 10 Pages: 3176-3181 Published: 2011

11 **Urinary excretion of low-molecular-weight proteins as prognostic markers in IgA nephropathy**

Peters, H. P. E.; van den Brand, J. A. J. G.; Wetzels, J. F. M.

Netherlands Journal of Medicine Volume: 67 Issue: 2 Pages: 54-61 Published: 2009

Graduate training overview

Name PhD student: AJG van den Brand **Department: Nephrology** **Graduate School: Radboud Institute for Health Sciences**	**PhD period: 1-4-2010 – 22-1-2015** **Promotor(s): JFM Wetzels** **Co-promotor(s): Dr. JM Hofstra**	
	Year(s)	ECTS
Training Activities		
Courses & Workshops		
Winterschool Dutch Kidney Foundation	2010	1.0
Genetic Epidemiology – Radboudumc	2010	5.7
BROK course - Radboudumc	2010	1.4
Start qualification in education - Radboudumc	2010	0.3
Basic qualification in education: Research Internships – Radboudumc	2010	1.0
NCEBP (now RIHS) Introduction course	2010	1.4
Academic Writing – Radboud University	2010	2.9
Personal Profile building – Radboud in'to Languages	2012	0.3
Nephrology Dialysis Transplantation: Reviewers-to-be	2012	1.4
Erasmus Winter Programme: Advanced Topics in Decision Making in Medicine – Erasmus MC	2013	1.4
Writing a successful ZonMW grant application – ZonMW and Radboudumc	2013	0.3
Scientific Integrity – Radboudumc	2013	1.4
Molecular Epidemiology of Chronic Diseases – M2E2 Maastricht	2014	2.0
Seminars & lectures		
American Society of Nephrology (ASN): Glomerulonephritis update	2011	0.6
Amgen ASN Review 2012 (oral presentation)	2012	0.3
European Renal Association – European Dialysis and Transplantation Association (ERA-EDTA) CME course: GFR2013 (invited lecture)	2013	0.3
Seminar on Research Integrity / Inaugural Lecture Prof. Dr. L Bouter – VUmc	2014	0.1

Symposia & congresses		
ASN Renal Week 2010 (oral presentation)	2010	1.4
NCEBP PhD Retreat 2011	2011	0.4
ASN Kidney Week 2011 (poster presentation)	2011	1.4
Dutch Federation for Nephrology: Nephrology days 2012 (oral presentation)	2012	0.3
NCEBP PhD Retreat 2012	2012	0.4
Dutch Federation for Nephrology: Scientific meeting (oral presentation)	2012	0.3
ASN Kidney week 2012 – (oral and 2 poster presentations)	2012 2013	1.4 0.3
NCEBP Science Day 2013	2013	0.4
NCEBP PhD Retreat 2013	2014	0.3
Symposium Scientific Integrity – VUmc	2014	1.0
ERA-EDTA convention 2014	2014	0.3
RIHS science day – (oral presentation)	2014	0.3
International symposium: GFR assessment in the year 2014 – Charité, Berlin, Germany (invited lecture)	2014	1.4
ASN Kidney Week 2014		
Other		
Journal club: Junior Researchers Epidemiology (weekly)	2010-2014	5.7
Journal club: Department of Internal Medicine (monthly)	2010-2014	2.9
Periodic lectures: Research meeting for the Department of Nephrology	2012-2014	1.4
Journal club: Department of Nephrology	2010-2014	1.4
Teaching Activities		
Lecturing		
Supervision of group assignments:		
writing a research proposal	2012-2014	1.0
body measurements	2013	0.1
Supervision of internships / other		
E Raho – Correcting kidney function for body composition	2011	2.0
MJE Verhoef – Invloed van albumine synthese, -filtratie en –resorptie op serum en urine albumine	2012	2.0
M van Rijn – Mediation analysis to identify successful components of a multifaceted treatment in chronic kidney disease	2014	3.5
Total		**51.6**

Curriculum vitae

Jan van den Brand werd geboren op 30 oktober 1984 te Eindhoven. Hij behaalde in 1997 zijn VWO diploma aan het gymnasium Bernrode te Heeswijk-Dinther. Daarna startte hij met de bachelor opleiding Biomedische wetenschappen aan het Universitair Medisch Centrum Sint Radboud in Nijmegen. Hij verrichte gedurende deze opleiding een stage bij het Nederlands Instituut voor onderzoek van de gezondheidszorg (NIVEL) in Utrecht onder leiding van prof dr Walter Devilé. Vervolgens volgde hij de masteropleiding Biomedische wetenschappen met als hoofdvak epidemiologie en bijvak International Health en een consultancy profiel. Tijdens de masteropleiding heeft hij stage gelopen bij het Muhimbili University College in Dar-es-Salaam, Tanzania. Als hoofdvakstage nam hij deel aan het onderzoek van Hilde Peters, onder supervisie van prof dr Jack Wetzels, hetgeen uitmondde in de publicatie *Urinary excretion of low-molecular-weight proteins as prognostic markers in IgA nephropathy*. Als laatste liep hij stage bij Integraal Toezicht Jeugdzaken, een samenwerkingsverband van vijf rijksinspecties. Binnen deze stage voerde hij een beleidsonderzoek uit naar de aanpak van overgewicht onder kinderen en jongeren. Zijn masteropleiding, tevens opleiding tot epidemioloog A, rondde hij in 2009 met goed gevolg af. Vanaf april 2009 werkte hij deels bij Integraal Toezicht Jeugdzaken als data analist en inspecteur, en deels als junior onderzoeker bij de afdeling nierziekten van het UMC Sint Radboud. In april 2010 is hij gestart met zijn promotieonderzoek, hetgeen gesteund werd door een aan Jack Wetzels toegekende subsidie van Nierstichting Nederland. Jan is getrouwd met Kim Bunthof.

Appendices

Supplementary information for the articles presented in this thesis

Supplements for Chapter 3.1: Low molecular weight proteins as prognostic markers for progression in idiopathic membranous nephropathy

Supplementary Table 3.1.1. Test characteristics of urinary α1- and β2-microglobulin excretion ratios.

Threshold value	sensitivity	specificity	PPV	NPV	false positives	false negatives	test positives
β2m							
≥ 0.5 mg/10 mmol creat	80%	67%	68%	79%	23	12	71
≥ 1.0 mg/10 mmol creat	75%	73%	76%	72%	16	17	68
≥ 1.5 mg/10 mmol creat	65%	83%	76%	73%	12	21	51
≥ 2.0 mg/10 mmol creat	58%	83%	74%	70%	12	25	47
≥2.5 mg/10 mmol creat	55%	84%	75%	68%	11	27	44
α1m							
≥ 75 mg/10 mmol creat	65%	78%	72%	72%	15	21	54
≥ 83 mg/10 mmol creat	58%	81%	73%	69%	13	25	48
≥ 100 mg/10 mmol creat	47%	87%	76%	65%	9	32	41
≥ 113 mg/10 mmol creat	45%	93%	84%	66%	9	32	41
≥ 125 mg/10 mmol creat	43%	94%	87%	66%	4	34	38

Excretion is expressed per 10 mmol creatinine in the urine. PPV: positive predictive value. NPV: negative predictive value. Test positives are the number of patients with a urinary α1- / β2-microglobulin excretion greater than the threshold value.

Supplementary Table 3.1.2. Regression coefficients of the predictive model for the progression of idiopathic membranous nephropathy.

	Regression coefficient	95% Confidence Interval		
Model 1: $u\beta_2m$				
$\ln(u\beta_2m)$	0.54	0.22	-	0.86
ln(screat)	3.06	0.33	-	5.8
ln(schol)	2.53	1.00	-	4.05
Intercept	-18.82	-32.10	-	-5.53
Model 2: $u\alpha_1m$				
$\ln(u\alpha_1m)$	1.19	0.49	-	1.91
ln(screat)	3.67	1.05	-	6.28
ln(schol)	2.32	0.81	-	3.83
Intercept	-25.59	-37.89	-	-13.28

ln: natural logarithm ($^e\log_n$), $u\beta_2m$: urinary excretion of β_2-microglobulin (µg/min), $u\alpha_1m$: urinary excretion of α_1-microglobulin (µg/min), screat: serum creatinine (µmol/l), schol: serum cholesterol (mmol/l).

Supplementary Table 3.1.3. Classification of patients according to uβ2m, outcome and tubulo-interstitial lesions.

Classification	Tubulo-intersitial lesions				Total
	None	Few	Moderate	Severe	
No progression, uβ2m < 1.0 µg/min	9	10	1	0	20
No progression, uβ2m ≥ 1.0 µg/min	1	4	0	0	5
Progression, uβ2m < 1.0 µg/min	4	2	1	0	7
Progression, uβ2m ≥ 1.0 µg/min	0	7	6	2	15
Total	14	23	8	2	47

Supplementary Table 3.1.4. Patient characteristics at baseline and repeat measurement.

Variables	Baseline	Repeated Measurement	Total study population	p
n (% male)	44 (64%)	44 (64%)	129 (68%)	0.58
age at time of biopsy (years)	49 (38 - 59)	49 (38 - 59)	51 (43 – 61)	0.25
time between biopsy and urine analysis (months)	2 (1 - 3)	12 (8 - 15)	2 (1 – 4)	0.74
Survival time (months)	29 (15 - 53)	29 (15 - 53)	25 (13 – 51)	0.55
MAP (mmHg)	95 (85 - 107)	89 (84 - 102)	97 (86 – 106)	0.72
Laboratory				
serum creatinine (µmol/l)	85 (75 - 95)	97 (81 - 113)	88 (76 – 103)	0.19
serum albumin (g/l)	23 (19 - 28)	25 (20 - 30)	23 (19 – 28)	0.93
serum cholesterol (mmol/l)	7.8 (6.2 - 9.4)	6.0 (5.0 - 7.3)	7.3 (5.7 – 9.2)	0.26
eGFR-MDRD4 (ml/min/1.73m2)	77 (66 - 91)	68 (55 - 82)	75 (60 – 87)	0.32
Urine samples:				
proteinuria (g / 10 mmol creatinine)	7.6 (5.1 - 10.4)	6.8 (4.3 - 10.4)	8.0 (5.6 – 10.7)	0.41
β2-microglobulin (µg/min)	0.5 (0.1 - 1.4)	1.1 (0.3 - 2.9)	0.6 (0.2 – 4.8)	0.05
α1-microglobulin (µg/min)	33 (21 - 45)	30 (15 - 69)	41 (23 – 72)	0.10
IgG (mg/24h)	229 (121 - 349)	182 (92 - 341)	257 (116 – 490)	0.07
Outcomes				
Progression	55%	55%	47%	0.36
50% rise in serum creatinine (n)	13	13	30	
25% rise and serum creatinine ≥ 135 µmol/l (n)	8	8	24	
clinical progression (n)	3	3	6	
Spontaneous remission			47%	0.46
partial remission:< 2.0 g / 10 mmol	41%	41%	61	
partial remission: <3.5 g / 10 mmol and ≥50% reduction	41%	41%	63	
complete remission	16%	16%	26	

Data are presented as median (interquartile range). MAP: mean arterial pressure. ACEi: angiotensin converting enzyme inhibitor. ARB: angiotensin-II receptor blocker. eGFR-MDRD4: estimated GFR calculated with the abbreviated MDRD formula. P values are given for the comparison of baseline characteristics for the total population and the 44 patients with repeated measurements. T- or X^2 tests were used.

Supplementary Table 3.2.5. Baseline characteristics of patients with idiopathic membranous nephropathy by year of referral.

Variables	**1995-2002**	**2003-2008**	**p**
n (% male)	58 (81%)	71 (58%)	0.005
age at time of biopsy (years)	49 (38 – 60)	56 (44 – 63)	0.09
time between biopsy and urine analysis (months)	2 (1 – 6)	2 (1 – 3)	0.01
MAP (mmHg)	98 (88 – 110)	97 (84 – 104)	0.08
Laboratory			
serum creatinine (µmol/l)	92 (79 – 113)	85 (76 – 95)	0.03
serum albumin (g/l)	23 (20 – 29)	24 (17 – 28)	0.10
serum cholesterol (mmol/l)	8.4 (6.9 – 9.7)	6.2 (5.3 – 8.1)	<0.001
eGFRMDRD4 (ml/min/1.73m2)	73 (58 – 87)	76 (64 - 87)	0.97
Urinary samples			
proteinuria (g / 10 mmol creatinine)	9.0 (5.6 – 11.0)	7.3 (5.3 – 10.7)	0.44
β2-microglobulin (µg/min)	0.5 (0.2 – 7.3)	0.7 (0.2 – 3.9)	0.14
α1-microglobulin (µg/min)	40 (18 – 72)	41 (23 – 80)	0.34
IgG (mg/24h)	296 (116 – 539)	232 (112 – 487)	0.64
Medication			
ACEi/ARB use at time of biopsy	31%	14%	0.03
Statin use at time of biopsy	19%	7%	0.05
Outcomes			
progression	57%	38%	0.03
partial remission:< 2.0 g / 10 mmol	40%	54%	0.12

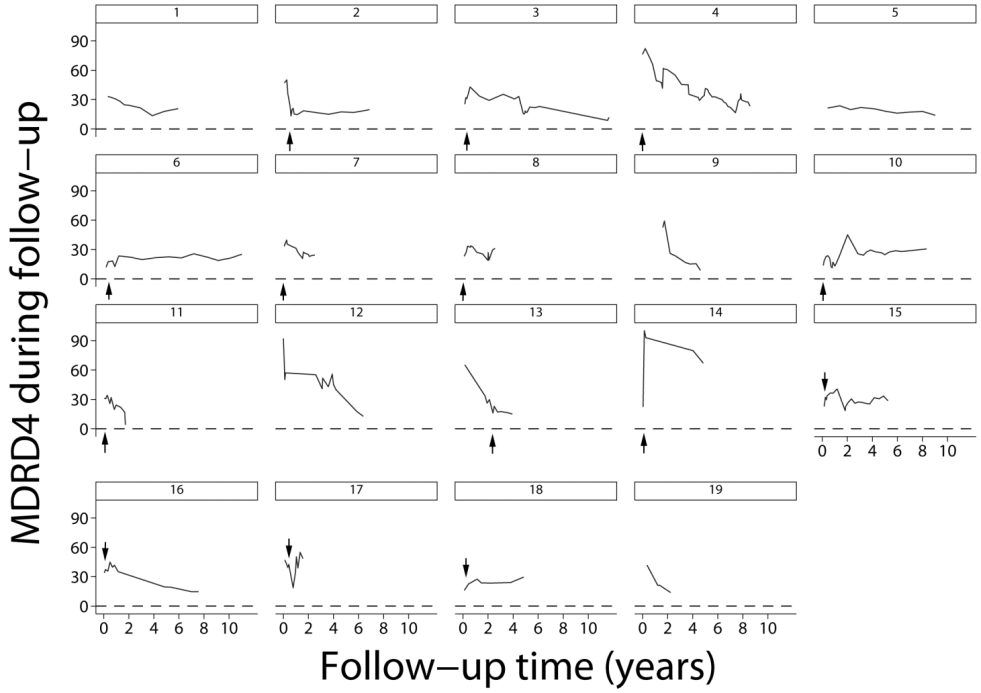
Data are presented as median (interquartile range). MAP: mean arterial pressure. The validation study by Branten et al (JASN 2005). included patients up to December 2002.

Supplementary Table 4.1.1: characteristics of patients showing severe kidney failure during follow-up.

Patient number	Gender	Age at baseline [years]	Immuno-suppressive Therapy	Remarks
1	male	65	none	Refused immunosuppression
2	male	72	CP	
3	female	67	CP	Third occurrence of relapse at end of follow-up
4	male	29	CP	Switched to azathioprine due to liver toxicity. Later switched to MMF, little effect on disease progression
5	female	66	none	
6	male	49	CP	
7	male	69	other IS	
8	male	59	other IS	
9	male	65	none	CP treatment recommended, refused due to expected adverse effects prednisone. Died due to cardiovascular causes.
10	male	64	other IS	
11	male	74	other IS	Treated with MMF, persistent proteinuria. Refused CP, died due to lymphoma.
12	female	67	none	Not treated due to significant comorbidity
13	female	40	CP	Initially refused immunosuppression, and thus delayed onset of therapy. Suspected noncompliant.
14	male	37	CP	
15	male	72	other IS	
16	male	55	other IS	MMF treatment terminated early due to infection

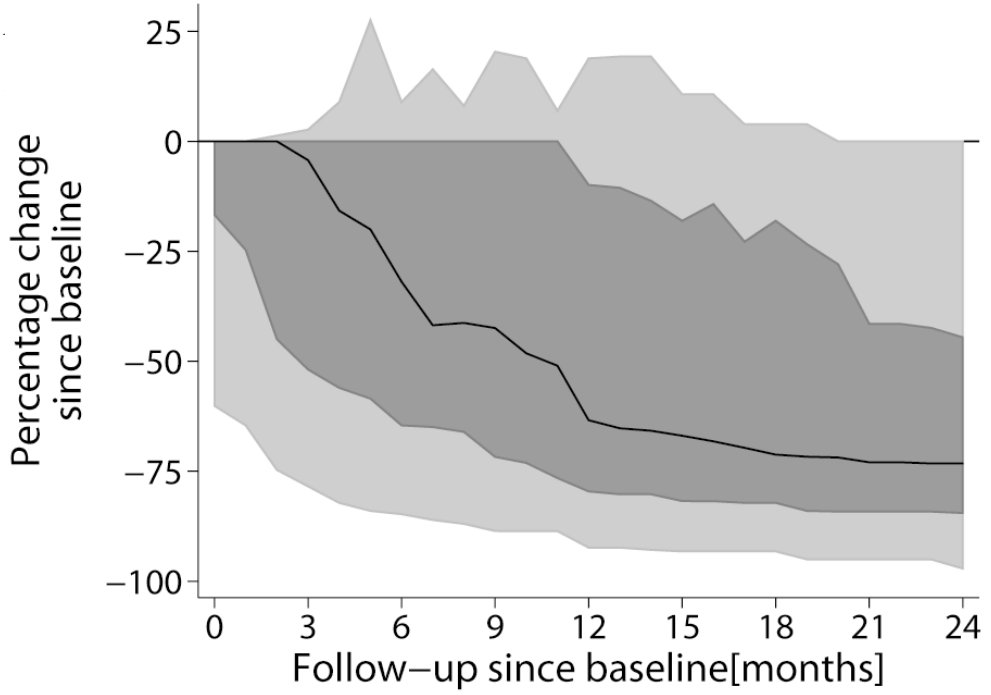
17	male	63	CP	
18	male	65	CP	
19	female	65	none	Suspected tubulointerstitial nephritis.
20	male	37	CP	CP treatment incomplete, switched to azathioprine, later cyclosporin both without success. Developed RRT
21	male	48	CP	Relapse after treatment with CP, later treated with MMF. Progressed to RRT.
22	male	70	none	AKI as a result of sepsis, leading to RRT
23	male	71	CP	Episode of acute kidney injury shortly after treatment was initiated. Required RRT
24	male	72	CP	Initially refused immunosuppression, required RRT.
25	male	59	CP	Therapy onset delayed due to infections. Persistent proteinuria, finally RRT
26	male	52	CP	Therapy recommended due to thrombo-embolic complication. However, therapy onset delayed. Massive proteinuria at end of follow-up. Required RRT.

CP: cyclophosphamide. IS: immunosuppression. MMF: Mycophenolate Mofetil. RRT: Renal Replacement Therapy



Supplementary Figure 4.1.1. Course of kidney function in patients who showed severe loss of kidney function but did not develop end stage kidney disease. Patients 3, 4, 7, 9, 11, 12, 13, 16 and 19 were considered to be at high risk of requiring renal replacement therapy in the near future. The arrow heads indicate the time treatment with immunosuppressive therapy was initiated.

Supplementary Figure S4.1.2. Relative change of proteinuria since baseline in



Supplements for Chapter 4.2: cancer risk after cyclophosphamide treatment in idiopathic membranous nephropathy

Full description of Patients and Methods

We included adult patients with biopsy proven idiopathic membranous nephropathy who visited our outpatient clinic between 1995 and 2009. Patients were either referred by a family doctor in our catchment area or by an allied centre. Secondary causes were ruled out per standard policy.1 Specifically, diagnostic procedures included chest X-rays, serology (ANA) to exclude systemic disease, and tests for hepatitis B and C. A mammography was performed in women over the age of 50 years and prostate specific antigen was obtained in men over 50 years of age. Additional investigations were undertaken if clinical suspicion for malignancy was raised by history, physical examination, other diagnostic test or, specifically, the presence of iron deficiency anemia. Written informed consent was obtained, and the study was conducted in accordance with the declaration of Helsinki and approved by the Radboud University Medical Centre medical ethics committee.

Most patients were treated according to our restrictive immunosuppressive regimen, detailed elsewhere.1 In summary, patients underwent a standardized, timed urine measurement.2 Subsequently, all patients received supportive treatment. This consisted of blood pressure control and proteinuria reduction with ACEi and/or ARBs, and further blood pressure lowering drugs to achieve target levels below 130/80 mmHg. Additionally, statins were given to treat hypercholesterolemia, and anti-coagulant therapy was considered in patients with severe hypoalbuminemia (<2.0 g/dl). In patients who reached a serum creatinine concentration above 1.5 mg/dL, immunosuppressive therapy was advised. Severe or life threatening symptoms of nephrotic syndrome were considered an indication to start treatment with immunosuppressive agents as well. Oral cyclophosphamide (1.5 mg/kg daily for twelve months) and pulse intravenous methylprednisolone (1 gram on days one to three, 61 to 63 and 121 to 123) in combination with high dose oral prednisone (0.5 mg/kg every other day for five months before tapering) was the preferred treatment. Occasionally, alternative immunosuppressive drugs were prescribed either as part of a clinical trial or when cyclophosphamide was contraindicated.3,4 In patients of reproductive age, the duration of cyclophosphamide therapy was reduced to three months, followed by nine months of azathioprine or mycophenolate mofetil. The resulting cumulative cyclophosphamide dose was less than 10 grams, which is considered safe to preserve fertility. From 1999 onward, trimethoprim-sulfamethoxazole was added to the regimen to prevent pneumocystis jiroveci pneumonia.

The outcome for the present study was incident malignancy, recorded from medical records and including the date of diagnosis. Mortality and the date of the final consultation were recorded as well

We pre-specified potential confounders, being age at time of biopsy, gender, ever smoking, having a first degree relative with a history of malignancy, chronic kidney disease stage (CKD, including substages 2a/b and 3a/b) and presence

of nephrotic syndrome at the time of biopsy. Immunosuppressive therapy was considered a possible confounder if it was initiated prior to cyclophosphamide therapy. Immunosuppressive therapy after cyclophosphamide could have acted as an intermediary, and thus adjustment could result in underestimation of possible malignancy risk. Gender, date of birth and height were recorded during urinary analysis at our center, whereas biopsy and follow-up laboratory data were obtained from medical records by two of the authors (PvD and JvdB). We recorded family history, smoking history, cyclophosphamide exposure (including total cumulative dose) and the use of other immunosuppressive drugs over the entire follow-up duration, including immunosuppressive drugs after cyclophosphamide treatment. Specifically we registered chlorambucil, azathioprine, mycophenolate mofetil, ciclosporin A, tacrolimus, methotrexate, prednisone and/or methylprednisone and experimental drugs (e.g. synthetic adrenocorticotropic hormone or rituximab), were obtained from medical records as well.

Statistical methods

Baseline data are presented as mean ± standard deviation (SD), median and inter quartile range (IQR) or frequencies and proportions. X2 test was used to evaluate differences in frequencies. The difference in means for normally distributed variables was compared using t-test. Wilcoxon's rank sum test was used to compare medians for the skewed variables.

Person time was calculated as the time from start of therapy until the occurrence of malignancy or the end of follow-up in the cyclophosphamide exposed group. Ideally, one would like to start measuring person time for controls at the moment that they would have started treatment. To mimic this moment of exposure, the median time between biopsy and initiation of therapy in the cyclophosphamide group was estimated and deducted from the time between biopsy and malignancy or end of follow-up in the control group. Consequently, time during which the exposed group was not actually at risk for malignancy due to cyclophosphamide exposure was not falsely included in the control group. If controls had negative person time as a result, they were excluded from the analyses.

Subsequently, the cumulative incidence of malignancy was calculated, assuming mortality prior to the occurrence of cancer was competing with malignancy risk. Unadjusted incidence rates were calculated and used to estimate the incidence ratio (IR) of malignancy after cyclophosphamide exposure. In order to estimate latency between cyclophosphamide exposure to outcome, the incidence ratio of malignancy was calculated by two year strata of person time. If empty cells were encountered when calculating the within stratum incidence ratios, a single event was added to both the cyclophosphamide and unexposed group. Potential confounding was investigated by stratifying according to age, gender, ever-smoking, prior immunosuppressive therapy , family history of malignancy, CKD stage and presence of the nephrotic syndrome. Age was categorized as under 44, 45 to 54, 55 to 64, 65 to74 and over 75 years. Standardized incidence ratios (SIR) were calculated by weighting for the distribution of the potential confounders in the cyclophosphamide treated group, as described by Rothman, Greenland and Lash.5 Note that no events were added to empty cells when SIRs were calculated.

Multiple imputation by chained equations was used to impute missing data on smoking status, family history and cumulative cyclophosphamide dose. Data on

malignancies, age, gender and prior immunosuppressive therapy were complete and used in the imputation model, as these variables were also considered for the final analysis model. In addition, baseline serum creatinine, serum albumin, mean arterial pressure, body mass index and baseline presence of nephrotic syndrome were included. The natural logarithm of continuous variables was taken in order to stabilize variance. Forty imputations were created using logistic regression for smoking status and family history, and linear regression for cumulative dose. The imputed data was checked visually using scatter plots. Poisson regression was used to obtain a multiple adjusted IR for the association between cyclophosphamide and malignancies, whilst taking the imputations into account. In addition, the dose-response relation between cumulative cyclophosphamide exposure and the occurrence of malignancy was investigated by creating 20 gram categories of cumulative exposure and including these in an adjusted Poisson regression. For all of the analyses above, 95% confidence intervals around the incidence ratios have been calculated.

Membranous nephropathy can occur secondary to cancer and can be incorrectly classified as idiopathic when that cancer is not yet detected. These patients are unlikely to respond to conventional therapy, and thus more likely to receive cyclophosphamide. As a result the association between cyclophosphamide and cancer (especially early cancers) may be inflated. Therefore, we checked for serum antiphospholipase A2 receptor antibodies (anti-PLA2R) at the time of referral, assuming that, if patients with early malignancies were predominantly seronegative for anti-PLA2R, the association between early cancers and cyclophosphamide would be the result of previously undiagnosed cancers. The serum samples were obtained at the time of urine analysis and stored at -80°C. Anti-PLA2R status was measured using a commercially available indirect immunofluorescence test (Euroimmun AG, Lübeck).

In sensitivity analyses, a Poisson regression was performed using only patients with complete data for smoking and family history. Secondly, analyses were repeated excluding patients who had received immunosuppressive drugs other than cyclophosphamide. Finally, age and gender cancer specific incidence in the present cohort was standardized to the general population using incidence estimates obtained by the Netherlands Cancer Registry over the past decade.6 To do so, person time was stratified according to age and gender. The expected number of cases within each stratum was calculated. Subsequently, a standardized incidence ratio was obtained by dividing the observed by the expected number of cases.

References

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Supplementary results

Supplementary Table 4.2.1. Immunosuppressive therapy with drugs other than cyclophosphamide by cyclophosphamide exposure.

Timing of treatment	Cyclophosphamide exposed (n=127)		Not exposed to cyclophosphamide (n=145)	
Initial treatment* (before CP)	Chlorambucil	5	Chlorambucil	1
	MMF	11	MMF	9
	ACTH	4	ACTH	7
	High dose corticosteroids (Coggins)	7	High dose corticosteroids (Coggins)	4
			Aza	1
Total initial treatment	27 (21%)		22 (15%)	
Additional treatment* (after CP)	MMF	13		
	ACTH	2		
	Aza	7		
	CsA	2		
	ACTH & Tacrolimus	3		
	ACTH & MMF	1		
	CsA & Aza	1		
	MMF & Aza	2		
	MMF, Tacrolimus & Aza	1		
Total secondary treatment	32 (25%)			

*MMF: mycophenolate mofetil, ACTH: synthetic Adrenocorticotropic Hormone, Aza: azathioprine, CsA: cyclosporin A.*Ten patients have received other immunosuppressive therapy both prior to and after cyclophosphamide therapy and have been counted double in this table. The median duration of cyclophosphamide therapy was 12 (IQR 12 to 16) months in cyclophosphamide treated patients. Patients treated with other immunosuppresion, and not cyclophosphamide, were treated for a median of 9 (IQR 5 to 12) months. Patients who received both CP and other immunosupression were treated with cyclophosphamide for 12 (IQR 6 to 15) months and other drugs for 12 (IQR 8 to 22) months, resulting in a total treatment duration of 27 (IQR 17 to 59) months. Coggins' regimen consists of high dose oral prednisone for 8 weeks. Chlorambucil was given as part of the Ponticelli regime (0.2 mg/kg daily orally). MMF was given as part of a clinical trial, if patients had a pregnancy wish or if they experienced severe side effects of cyclophosphamide. The regular MMF dose was 500mg to 1000mg twice daily orally for 9 to 12 months. ACTH was administered for nine months during a clinical trial (clinicaltrials.gov: NCT00694863). Patients received 1 mg by intramuscular injections in increasing dose with a maximum of 1 mg twice weekly. Azathioprine was given in case of side effects of*

cyclophosphamide. Dose was usually 100mg per day. It was decreased to 50 mg per day over time in most patients and stopped after a year of treatment. Cyclosporin A was occasionally given if patients refused alkylating agents, dosage varied between 100 and 300mg daily. Tacrolimus was given instead of cyclosporin in more recent years.

Sensitivity analyses

Patients who received immunosuppressive drugs other than cyclophosphamide were excluded. Of the cyclophosphamide treated patients, 17 received other immunosuppression prior to cyclophosphamide, 22 received other immunosuppression afterwards, and 10 patients received other immunosuppression both before and after cyclophosphamide treatment. In total, 201 patients were included, 78 were treated with cyclophosphamide, 123 patients were not treated with immunosuppression at all. The population characteristics are presented in the table below. We then performed univariate, standardized and multiple adjusted analyses, the latter using Poisson regression. The adjusted analyses were preformed after multiple imputation of missing values for family history and smoking. Table 3 shows the incidence ratio ratios for cancer after cyclophosphamide exposure: unadjusted, standardized to potential confounders and after multiple adjustment.

Supplementary Table 4.2.2: Baseline population characteristic after exclusion of patients treated with other immunosuppressive drugs.

Variables	Cyclophosphamide treated (n=78)	Not treated with cyclophosphamide (n=123)	P
At time of biopsy			
males (n, %)	61 (78%)	77 (63%)	0.02
BMI (kg / m2)	26.9 ± 3.6	27.2 ± 5.2	0.58
age (years)	56.1 ± 11.1	49.2 ± 14.7	<0.001
year of biopsy	2001 ± 5.1	2002 ± 6.5	0.81
follow-up duration (years)	6.0 (3.7 - 10.7)	5.5 (2.9 - 8.4)	0.73
positive family history for malignancy*	9/52 (17%)	10/84 (12%)	0.38
current/former smoker*	37/61 (61%)	49/89 (55%)	0.46
eGFR-MDRD4 (ml/min/1.73m^2)	62 ± 21	77 ± 20	<0.001
serum creatinine (mg/dl)	1.2 (1.0 – 1.5)	0.9 (0.8 – 1.1)	<0.001
serum albumin (g/dl)	2.0 (1.7 – 2.5)	2.8 (2.4 – 3.1)	<0.001
nephrotic syndrome at presentation	75 (96%)	100 (81%)	0.002
protein : creatinine ratio (g / g)	8.8 (5.9 – 11.1)	4.5 (2.7 – 6.6)	<0.001
ACEi/ARB use	68 (87%)	113 (92%)	0.28
statin use	49 (63%)	73 (59%)	0.62
other BP lowering medication	24 (31%)	17 (14%)	0.004
Therapy			
interval from baseline to start of therapy (months)	9 (4 - 16)	n/a	.
serum creatinine at start of therapy (mg/dl)	1.4 (1.1 – 1.9)	n/a	.
cumulative cyclophosphamide dose (g)	37 (27 - 46)	n/a	.
Outcomes			
death	11 (14%)	6 (5%)	0.02
malignancies	11 (14%)	3 (2%)	0.002

**The denominator differs from the total number of patients due to missing data for this variable. Data are presented as mean ± standard deviation, median (inter quartile range) or percentages where appropriate. BMI: Body mass index; eGFR-MDRD4: estimated glomerular filtration rate, calculated with the abbreviated Modification of Diet in Renal Disease equation for mass spectrometry standardized creatinine; ACEi: angiotensin converting-enzyme inhibitor; ARB: angiotensin II receptor blocker; BP: blood pressure.*

Supplementary Table 4.2.3: Incidence ratios for malignancy after cyclophosphamide exposure by possible confounders. Analysis limited to patients without immunosuppression other than cyclophosphamide.

Risk Factor	Incidence Ratio	95% Confidence Interval		
Unadjusted	6.3	1.7	-	35.1
Univariate adjusted:				
Age	3.8	0.8	-	17.1
Male gender	4.6	1.3	-	16.7
Smoking	5.3	1.4	-	19.9
Family history of malignancy	7.4	1.6	-	35.0
CKD stage	10.3	2.8	-	38.0
Nephrotic syndrome	6.2	1.4	-	27.3
Multiple adjusted*	5.1	1.2	-	21.5

**After multiple imputation, complete case analysis: (n=150; IRR=3.4 [95%CI: 0.8 - 14.9]). The analysis was adjusted for age, gender and ever smoking in both the imputed and complete case analyses.*

Supplementary Table 4.2.4. Incidence ratio of malignancy for the total cohort, cyclophosphamide exposed and unexposed patients standardized to the general Dutch population stratified by gender and age.

			Total cohort			Cyclophosphamide exposed			Unexposed		
Gender	Age	Average Population Incidence (per 100,000 persons per year)	Person time (years)	observed cases	expected cases	Person time (years)	observed cases	expected cases	Person time (years)	observed cases	expected cases
Females	15-19	31.3	6.4		0.00				6.4		0.00
	20-24	57.4	14.9						14.9		
	25-29	104.7	27.8		0.03	2.1		0.00	25.7		0.03
	30-34	169.0	35.8		0.06				35.8		0.06
	35-39	280.0	42.3		0.12	2.7		0.01	39.6		0.11
	40-44	453.0	51		0.23	11.3		0.05	39.7		0.18
	45-49	640.6	58.5	1	0.37	15.4	1	0.10	43.1		0.28
	50-54	801.9	49.3		0.40	11.9		0.10	37.4		0.30
	55-59	1035.7	65.3		0.68	18.4		0.19	46.9		0.49
	60-64	1286.8	58.6		0.75	30.4		0.39	28.2		0.36
	65-69	1535.6	55.5	2	0.85	32.3	2	0.50	23.2		0.36
	70-74	1663.1	28.9		0.48	16.5		0.27	12.4		0.21
	75-79	1904.7	11.6		0.22	5		0.10	6.6		0.13
	80-85	1942.4	5.5		0.11	3.3		0.06	2.2		0.04
Males	15-19	37.5	13.7		0.01				13.7		0.01
	20-24	53.7	19.7		0.01	2.8		0.00	16.9		0.01
	25-29	68.4	34.1		0.02	18.4		0.01	15.7		0.01
	30-34	87.3	56.2		0.05	30.1		0.03	26.1		0.02
	35-39	126.2	83.6		0.11	40.3		0.05	43.3		0.05
	40-44	217.4	110.3	1	0.24	46.4		0.10	63.9	1	0.14
	45-49	410.1	139.4		0.57	84.6		0.35	54.8		0.22
	50-54	762.2	158.6	1	1.21	110.4	1	0.84	48.2		0.37
	55-59	1307.2	141.4	1	1.85	83.1	1	1.09	58.3		0.76
	60-64	2015.1	133.8	2	2.70	64.1	2	1.29	69.7		1.40
	65-69	2744.5	131.9	3	3.62	69.7	2	1.91	62.2	1	1.71
	70-74	3392.7	59.8	2	2.03	35.4	1	1.20	24.4	1	0.83
	75-79	3660.0	26.6	4	0.97	16.4	3	0.60	10.2	1	0.37
	80-85	3555.4	9.0	3	0.32	9.0	3	0.32			
				20	17.7		16	9.2		4	8.4
SIR				1.13			1.73			0.47	
lower				0.64			0.88			0.01	
upper				1.63			2.58			0.94	

SIR: Standardized Incidence Ratio. Lower and upper represent the lower and upper limit of the 95% confidence interval, respectively. Person time distributed over the strata according to ageing. For example if a 42 year old male had a 12 year follow-up, 3 person years would contribute to person time in the age stratum 40 to 44, 5 years to the stratum 45 to 49 and the final 4 years to the stratum 50 to 54.

Supplements for Chapter 5: Cost-effectiveness of a restrictive treatment strategy in idiopathic membranous nephropathy

Distributions

Supplementary Table 5.1. Model input: transition probabilities for the Markov process.

Transitions	Calculations	Distribution	Parameters
Cancer after dialysis	Population cancer risk (look up table 5) x standardized incidence ratio (SIR)	normal	mean: ln(1.4), sd: (ln(1.5)-ln(1.3))/4
Cancer following cyclophosphamide therapy	Population cancer risk (look up table 5) x SIR for CP treated iMN patients	normal	mean: ln(1.7), sd: (ln(2.6)-ln(0.9))/4
Cancer following renal transplantation	Population cancer risk (look up table 5) x SIR for cancer in NTx	normal	mean: ln(3.3), sd: (ln(3.5)-ln(3.1)) /4
Cancer without cyclophosphamide (baseline risk)	Population cancer risk (look up table 5) x SIR for iMN patients	normal	mean: ln(0.5), sd: (ln(0.9)–ln(0.1))/4
Complications due to cyclophosphamide therapy	n/a	Beta	Events: 35, N: 91
Complications due to the nephrotic syndrome	Three year risk, converted to a rate and divided by 3.	Beta	Events: 65, N: 898
Complications while in remission (base risk)	n/a	Poisson:	5.0/10 000
Death after dialysis	Look-up table 3		
Death after renal transplantation	Look-up table 1		
Death due to cancer	Look-up table 2		
Death due to cancer after dialysis	n/a	Beta	Events:151, N:178
Death due to cancer after NTx	n/a	Beta	Events: 94, N:150
Death due to complications	Ten year risk converted to rate and divided by 10	Beta	Events: 15, N: 254
Death while in remission	Look-up table 6		

Death while suffering from the nephrotic syndrome	Mortality risk for controls in Ponticelli trial	normal	mean: ln(2.8) sd: (ln(9.7)-ln(0.8))/4
ESRD when suffering from the nephrotic syndrome	ESRD risk for controls in Ponticelli trial (risk converted to a rate and divided by 10)	Beta	Events: 9, N:26
Graft loss after kidney transplantation	Look-up table 1		
Progressive loss of kidney function	Annual probability of progression (and thus treatment) when uβ2m is false negative	Beta	Events: 23, N:47
Receiving cyclophosphamide treatment in the prolonged watchful waiting strategy	Risk of kidney function loss within 3 years	Beta	Events: 58, N:129
Relapsing nephrotic syndrome	n/a	Beta	Events: 99, N:254
Remission following cyclophosphamide therapy	Remission in the treated group of the Ponticelli trial	Beta	Events: 26, N:42
Spontaneous remission of proteinuria	Remission in the control group of the Ponticelli trial	Beta	Events: 4, N:39
Transplantation after dialysis has been initiated	Look-up table 4		
Sensitivity of $u\beta_2m$	n/a	Beta	Events: 24, N: 24
Specificity of $u\beta_2m$	n/a	Beta	Events: 17, N: 20
Probability that $u\beta_2m$ test is positive (high risk)	n/a	Beta	Events: 27, N: 44

Supplementary Table 5.2. Model input: rewards for Markov transitions

State reward	Distributions	Parameters
Utilities		
Cancer	triangular	likely: 0.75, min: 0.70, max: 0.92
Complications	triangular	likely: 0.70, min: 0.50, max: 0.87
Death	n/a	0
Dialysis	triangular	likely: 0.43, min: 0.43, max: 0.63
Graft loss	triangular	likely: 0.62, min: 0.62, max: 0.66
Kidney transplantation	triangular	likely: 0.84, min: 0.84, max: 0.94
Nephrotic Syndrome	normal	mean: 0.74 sd: 0.07
Remission	uniform	min: 0.95, max: 1.00
Utility while receiving CP treatment	triangular	likely: 0.84, min: 0.72, max: 0.94
Healthcare costs (€)		
Annual costs of dialysis	triangular	likely: 69554.00, min: 69554.00, max: 86083.00
Annual costs of kidney transplantation	triangular	likely: 13452.61, min: 13452.61, max: 35870.50
Cancer	triangular	likely: 8420.67, min: 1941, max: 23336.28
Complications	triangular	likely: 2194.86, min: 757.01, max: 3915.00
Conservative therapy	triangular	likely: 781.13, min: 781.13, max: 1098.84
Cyclophosphamide therapy	triangular	likely: 1982.82, min: 1982.82, max: 2022.43
Death	triangular	likely: 4042.08, min: 3024.42, max: 4474.44
Initial costs of kidney transplantation	triangular	likely: 24673.51, min: 15235.53, max: 85302.28
Initial costs of dialysis (also graft loss)	triangular	likely: 2521.54, min: 2479.44, max: 8855.72

Scenarios for cost calculation

Costs were obtained from the budget for the department of nephrology of the Radboud University Medical Centre, courtesy of drs A. van Lieshout, managing director. Medication costs were calculated using the Dutch Healthcare Insurance Board's price estimates (www.medicijnkosten.nl):

Death

We assumed a patient to be admitted to the intensive care unit two days prior to death. The minimal costs scenario entails an admission fee and two days stay without intubation and mechanical ventilation according to reimbursement group 1 (www.nza.nl). The likely cost scenario was admission and two days stay with intubation and mechanical ventilation within reimbursement group 2.

Finally, the maximum cost scenario was comparable to the likely cost scenario, except for reimbursement group 3.

Graft loss

We assumed that patients experiencing graft loss would receive anti-rejection therapy. Otherwise the costs are comparable to the initial costs of dialysis.

Initial costs of transplantation

The number of procedures performed have been taken from the 2012 budget for the department of nephrology of the Radboud University Medical Centre, courtesy of drs A. van Lieshout, managing director.

Procedure	minimal costs	likely costs	maximum costs
screening donor	616.78	2 535.21	5 127.17
screening recipient	1 175.87	1 175.87	3 0781.17
operation (nephrectomy and implantation)	8 386.01	15 905.56	23 595.02
Post-op admission recipient	4 035.57	4 035.57	19 906.43
Readmission within one year	8 086.97	8 086.97	3 6242.50
Outpatient follow-up	629.23	629.23	5 330.63
Total	€24 173.86	€24 427.17	€103 307.27

Medication	minimal costs	likely costs	maximum costs
Cotrimoxazol (480 mg dd 3 months)	9.42	9.42	10.62
Valganciclovir (450mg eod 2 months)	776.14	776.14	776.14
ceftriaxon (2000mg 1 time pre-op)	19.06	19.06	19.91
tacrolimus (15mg dd 1 year)	8 744.79	8744.79	1 0581.00
prednisone (8mg dd 1 year)	27.88	27.88	28.65
mycophenolate mofetil (2000 mg dd 6 months)	180.05	180.05	988.52
metoprolol (200mg dd 1 year)	15.14	15.14	23.79
Total	€9 772.48	€9 772.48	€12 428.63

Annual costs of transplantation

The annual hospital and physician costs of transplantation were assumed to be the comparable to readmissions and outpatient follow-up.

Procedure	minimal costs	likely costs	maximum costs
Readmission	4 035.57	4 035.57	19 906.43
Outpatient follow-up	629.23	629.23	5 330.63
Total	€4 664.80	€4 664.80	€25 237.06

Medication	minimal costs	likely costs	maximum costs
tacrolimus (15mg dd 1 year)	8 744.79	8 744.79	10 581.00
prednisone (8mg dd 1 year)	27.88	27.88	28.65
metoprolol (200mg dd 1 year)	15.14	15.14	23.79
Total	€8 787.81	€8 787.81	€10 633.44

Therefore the annual costs of kidney transplantation were estimated at: €13 452.61, with a minimum of €13 452.61 and a maximum of €35 870.50.

Cyclophosphamide therapy

The costs for cyclophosphamide therapy were calculated based on the following therapeutic regime: Patients were admitted to daycare for a methylprednisolone infuction 9 times 1000mg (days 1,2 and 3; 61,62 and 63; and 120,121 and 122). They received 150 to 200mg of cyclophosphamide daily for 6 months and 40mg of prednisone every other day for five months before tapering.

Procedure/medication	minimal costs	likely costs	maximum costs
admisstion to daycare + IV (9x)	1 540.90	1 540.90	1 540.90
methylprednisone (9x 1000mg)	298.17	298.17	298.17
cyclophosphamide (150 to 200mg dd 6 months)	108.91	108.91	147.55
prednisone (40mg eod 6 months)	34.84	34.84	35.81
Total	€1 982.82	€1 982.82	€2 022.43

Conservative therapy

Costs for conservative therapy were calculated based on 4 outpatient visits, treatment with an ACEi or ARB, a statin and a diuretic.

Procedure/medication	minimal costs	likely costs	maximum costs
Outpatient visits	763.50	763.50	763.50
ACEi /ARB (daily 1 year)	5.49[i]	5.49	151.37[ii]
Statin (daily 1 year)	6.49[iii]	6.49	172.95[iv]
Diuretic (daily 1 year)	5.65[v]	5.65	11.05[vi]
Total	€781.13	€781.13	€1098.84

[i] Enalapril 20mg, [ii] Irbesartan 75mg, [iii] Simavastatin 20mg, [iv] Atorvastatin 20mg, [v] Hydrochlorthiazide 25mg, [vi] Furosemide 20mg.

Initial costs of dialysis

Prior to the actual initiation of dialysis, patients will be monitored more closely. We assumed the initial costs of dialysis be consist of costs made during the predialysis phase.

Procedure/medication	minimal costs	likely costs	maximum costs
Predialysis outpatient visits	779.86	779.86	7093.00
Placing A/V shunt	63.16	105.26	126.32
Erythropoiesis stimulating agents	1 289.70	1 289.70	1 289.70
Calcium supplement	161.80	161.80	161.80
Vitamin D	184.92	184.92	184.92
Total	€2 479.44	€2 521.54	€8 855.72

Annual costs of dialysis

The annual costs of dialysis have been taken from the budget of the department of nephrology, Radboud University medical centre. 2 379 out of 3 093 (77%) registered claims were up to three dialysis sessions per week. These were thus taken as the minimum and likely costs. 540 (17%) reimbursement claims were four to five sessions per week. Other instances, such as in hospital dialysis were considered so rare (at most 57 claims) that these were not considered for cost calculation. The reimbursed costs include hospital, physician and medication costs.

Procedure	minimal costs	likely costs	maximum costs
Dialysis	€69 554.00	€69 554.00	€86 083.00

Complications

Complications considered for cost estimate were pneumonia, thrombosis, pulmonary embolism, CVA and CVD (including peripheral vascular disease). For the first two were considered potentially least costly, as these may be treated with antibiotics and without admitting the patient. The other three were considered reasons for admission.

Complication	minimal costs	likely costs	maximum costs
Pneumonia	118.83	366.38	366.38
Thrombosis	379.80	2 848.30	7 109.10
Pulmonary embolism	1 365.50	2 755.50	3 360.90
CVA	1 242.70	3 195.60	6 591.00
CVD	678.20	1 808.50	2 147.60
Mean	€757.01	€2 194.86	€3 915.00

Cancer

The most common forms of cancer in iMN patients are lung, lymphoma, leukemia, colon, prostate, mamma and bladder cancer. Costs for cancer diagnosis and treatment were calculated based on costs estimations for hospital in-patient, out-patient and daycare day. The estimated number of days per patient by disease were are presented in the table. Respective costs were €425 ±81, €111 ±50 and €201 ±82 per inpatient, outpatient and daycare day.

Lung cancer

The diagnosis, treatment and aftercare for lung cancer were based on the Dutch guidelines for small cell lung cancer. The diagnosis requires two outpatient visits for history, lab and imaging and a daycare visit for a lung biopsy. Treatment was considered to be chemotherapy with six doses of cis- or carboplatin requiring a daycare admission and etoposide 50 mg/m2 for three weeks per cycle. Aftercare consisted of four outpatient visits within the first year after treatment.
Hospital costs: 7 x €201 + 6 x €111 = €2073.00
Medication costs: 6x €204.79 + 6x €366.18 = €3425.82

Lymphoma and leukemia

The diagnosis of lymphomas and leukemia is assumed to require two outpatient visits and a daycare visit for biopsy. Treatment is assumed to be 6 cycles of R-CHOP21 for a patient with a body surface area of 1.8m2. Thus medication was 675mg rituximab, 1400 mg cyclophosphamide IV, 90 mg doxorubicin IV, vincristine 2 mg IV on the first day of each cycle and 100 mg prednisone on days 1 through 5. Aftercare required two outpatient visits in the first year.
Hospital costs: 7 x €201+ 4x €111 = €1851
Medication costs: 6x €3168.29 = €19009.74

Colon cancer

Diagnosis is by means of coloscopy and biopsy in an outpatient setting. Treatment consists of laparoscopic surgery (7 inpatient days assumed) and

adjuvant chemotherapy (FOLFOX: oxaliplatin 85 mg/m2 (€1222.49), folinic acid 200 mg/m2 (€8.79) and fluorouracil 1000 mg/m2 (€6.55) = €1237.83). Aftercare consists of two outpatient visits per year.
Hospital costs: 7 x €425 + 2 x €111 = €3197
Medication costs: €1237.83

Prostate cancer

Diagnosis of prostate cancer is assumed to require a biopsy, which can be performed in an outpatient setting. In the treatment of prostate cancer radical prostatectomy, TURP and TURT are considered the first line treatments. The average length of stay is assumed to be 3 days. Aftercare consists of 5 outpatient visits.
Hospital costs: 3 x €425 + 6 x €111 = €1941

Breast cancer

Diagnostic procedures consist of mammography, echography and biopsy all performed during an outpatient visit. Treatment was assumed to be restricted mastectomy and sentinel node resection, 3 days admission on average. Adjuvant chemo was considered as well (6 courses of TAC à €3443.88) in a daycare setting. Aftercare consists of annual mammography.
Hospital costs: 3 x €425 + 6 x €201 + 2 x €111 = €2703
Medication: 6x €3443.88 = €20663.28

Bladder cancer

Diagnosis requires cytologic investigation and cystoscopy, performed in the outpatient clinic. Treatment may consist of radical cystectomy (€1396) or TURP (€1265) and 3 days admission. Aftercare consists of two outpatient visits per year.
Hospital costs: €1265 or €1396 + 3 x €425 + 3 x €111 = €2873 to €3004

Estimated cost distribution for cancer

Cancer	Hospital costs	Medication	Total
Lung	€2 073.00	€3 425.82	€5 498.82
Lymphoma/leukemia	€1 851	€19 009.74	€20 860.74
Colon	€3 197	€1 237.83	€4 434.83
Prostate	€1 941	0	€1 941.00
Breast	€2 703	€20 663.28	€23 336.28
Bladder	€2 873	0	€2 873.00

Minimal costs: €1 941, mean costs €8 420.67, maximum costs: €23 336.28.

Look-up tables

Look-up table 1. Probability of death following kidney transplantation

Age	Probability of death		
	1 year after NTx	5 years after NTx	10 years after NTx
20-29	0.032	0.088	0.315
30-39	0.029	0.073	0.297
40-49	0.031	0.078	0.326
50-59	0.042	0.101	0.395
>60	0.064	0.155	0.558

Look-up table 2. Probability of death due to cancer (general population)

Age	Probability of death
20-44	0.09
45-54	0.17
55-64	0.21
65-74	0.26
>75	0.34

Look-up table 3. Death on dialysis

Age	Probability of death
20-64	0.03
65-74	0.11
>75	0.23

Look-up table 4. Probability of receiving a kidney transplantation (initial or after dialysis)

Age	Probability of receiving a kidney transplant
20-44	0.46
45-65	0.37
>65	0.24

Look-up table 5. Risk of cancer in the general population

Age	Probability of cancer
20-24	0.00056
25-29	0.00087
30-34	0.00128
35-39	0.00203
40-44	0.00335
45-50	0.00525
50-54	0.00782
55-59	0.01171
60-64	0.01651
65-69	0.02140
70-74	0.02528
75-79	0.02782
≥80	0.02749

Look-up table 6. Probability of mortality in the general population

Age	Probability of death
20-29	0.00055
30-39	0.00055
40-49	0.0011
50-59	0.0032
60-69	0.0079
70-79	0.0222
80-89	0.0646
≥90	0.1805